THE EFFECT OF ACIDIFICATION ON EPILITHIC ALGAE IN THE LOCH ARD CATCHMENT

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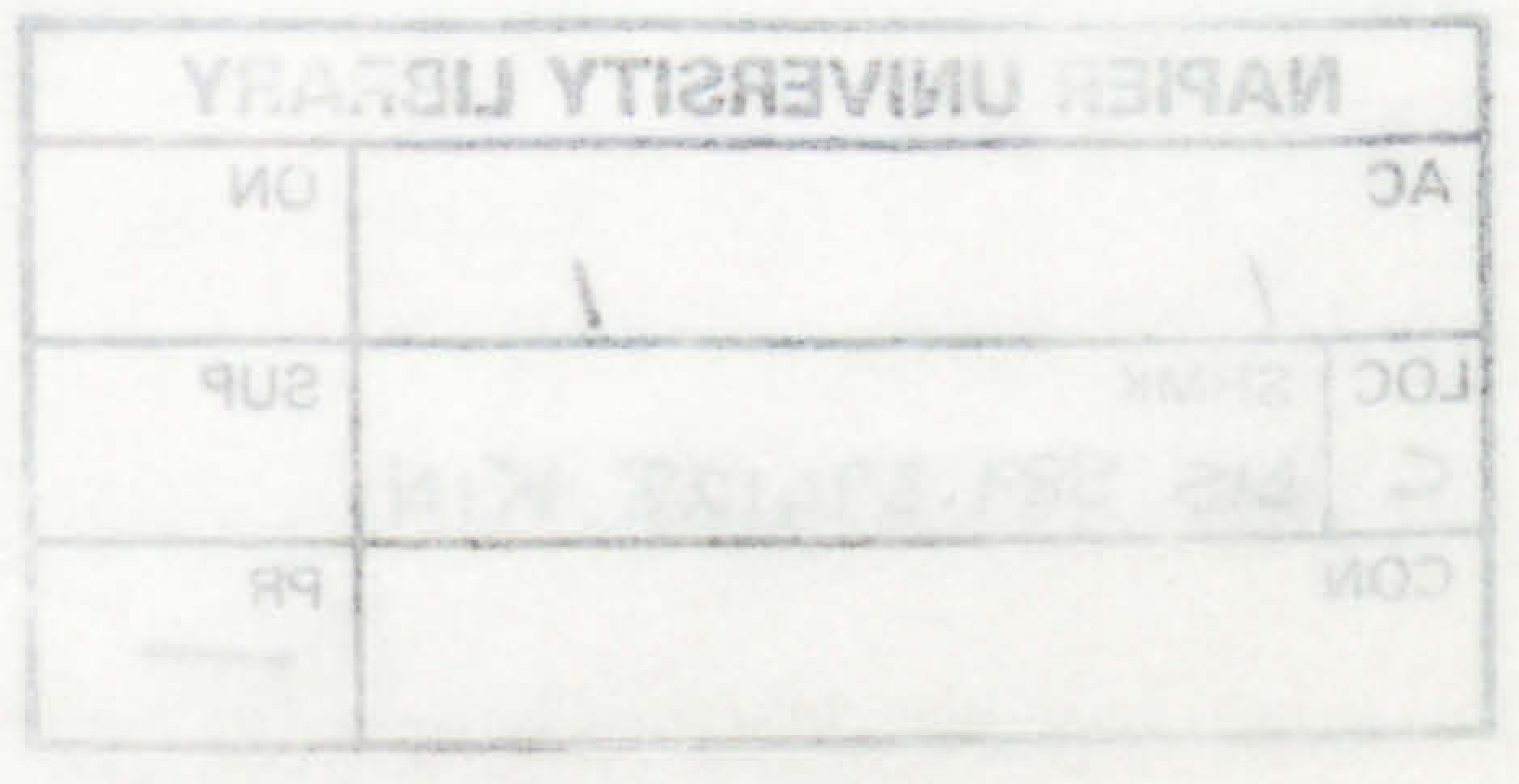

To Graham

DECLARATION

I hereby declare that:

All the work presented in this thesis has been carried out by myself and that no part of this work has been submitted in support of a degree validated by the CNAA or a University

John Kinross

(Signed)
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Glossary of abbreviations and terms used

A_x Optical Absorbance (Optical density units) at wavelength x nanometres

A_{250} comp. Absorbance at 250 nm of composite (1 week) water samples

AFDW Ash-Free Dry Weight (Weight loss on ignition at 550°C)

ALK Alkalinity

Al-TM Total Monomeric aluminium. Dissolved or complexed Al measured in catechol violet assay, not subjected to passage over ion-exchange resin

Al-NL Non-Labile aluminium. Dissolved Al complexed with organic or other ligands; does not bind to ion exchange resin

Al-L Labile aluminium; retained by ion exchange resin

ANOVA ANalysis Of VAriance

CANOCO Computer program performing CANOnical COrespondence analysis (Ter Braak, 1988)

CCA Canonical Correspondence Analysis

DCCA Detrended Canonical Correspondence Analysis. 'Detrending' is a means of removing any 'arch' in the data along axis 2

DIC Dissolved Inorganic Carbon

'Data form'

Species data is utilised in either 'abundance' (estimated relative abundance) or 'presence-absence' (p/a) form. For most applications mean abundance or 'pooled p/a' is calculated

HDPE High Density PolyEthylene

Matrix Species, biomass and environmental variables are measured in up to 15 sites, on up to 35 occasions, to give a 3 dimensional matrix, matrix C. Calculation of mean species/biomass or environmental variable values yields a 2 dimensional version. Division of the 3-D matrix along the 'occasions' dimension yields matrices A and B, the earlier and later parts of the sampling programme.

Matrix D consists of data collected in a preliminary survey in 1985 (Kinross, 1985)

NAA Normal Association Analysis, carried out by the program NASSOC.BAS (Ludwig and Reynolds, 1988). Sampling Units are sorted into groups on the basis of the species they contain. The χ^2 (chi-squared) value for each species pair in turn is computed. Yate's corrected chi-squared is also available which corrects for bias due to rare species. Presence-absence data are used.

IAA Inverse Association Analysis - sorting of species into groups on the basis of their presence or absence in different SUs. It is carried out using NASSOC.BAS on an inverted data matrix.

NO_x Collective term for gaseous oxides of nitrogen

PAR Photosynthetically Available (or Active) Radiation. Light in the wavelength band 300-700 nm. Measured as quanta (photons); units are micromoles per square metre per second (formerly microeinsteins per square metre per second). 1 mole = 6.025×10^{23} photons

PAR % Measured incident PAR expresssed as a percentage of that available in an 'open' unshaded site, i.e. the reference site; burn 6

PAR.ABS Absolute PAR - recalculated values of PAR at a site, in $\mu moles.m^{-2}s^{-1}$. Calculated from product of measured incident PAR at the reference site and the mean PAR% value for the secondary site

pH INST Instantaneous pH = pH measured in spot samples

pH COMP Composite pH = pH measured in composite samples

RUN Experiment carried out to measure growth rate in channels. Runs 1-3 involved pH manipulation, 4 and 5 involved different Al and Si concentrations

SOAFD Scottish Office Agriculture and Fisheries Department

Species Diversity

An expression of the degree to which a natural community is composed of different

species. It is composed of two components - Richness and Evenness (see section 4.4)

Richness - may be expressed by the number of species in a sample (a) as a mean value per site or (b) as the number of species found on each sampling occasion. Both versions have been examined in this study

SU Sampling Unit, the term used in statistical ecology (e.g. Ludwig and Reynolds, 1988) to refer to a discrete division of the environment subjected to a sampling procedure. It corresponds to a sampling site in this work

TOC Total Organic Carbon

TON Total Oxidized Nitrogen ($NO_3^- + NO_2^-$)

VIF Variance Inflation Factor. One statistic provided by the output from the program CANOCO; VIF values greater than approximately 20 imply that the environmental variable in question is highly correlated with others in the analysis.

SUMMARY

A survey of epilithic filamentous algae was carried out at 15 sites on 10 streams with a range of mean pH from 4.37 to 6.67 in the Loch Ard area of the Trossachs, between 1986 and 1988. Monitoring of physical and chemical parameters was carried out in parallel. Photosynthetically Available Radiation (PAR) was measured using electronic integrators developed during the course of the study.

Samples of epilithic algae were taken from natural and artificial substrates (microscope slides) to determine the relative contribution of different species to the community structure. Taxa could not be identified to species level in most cases, and are described by genus and cell diameter. Forty-nine taxa were distinguished in the filamentous algal communities found. Relative abundance of taxa was estimated. The mean value of abundance was calculated for use in subsequent statistical analyses. As an alternative, presence-absence (p/a) on each occasion was scored, and a mean value (pooled p/a) similarly calculated. Samples were taken also to determine algal biomass, as acetone or methanol extractable pigments (chlorophyll a and carotenoid) and ash-free dry weight (AFDW).

The community structure at the different sites was investigated using statistical ecology computer programs. Using Inverse Association Analysis and Canonical Correspondence Analysis, distinct species assemblages were found in sites with mean pH values towards the extremes of the range encountered. Using Normal Association Analysis, Cluster Analysis and Canonical Correspondence Analysis, it was shown that sites are separable on the basis of the species they contain into groups related by mean pH. Using the results of Canonical Correspondence Analyses carried out by the program CANOCO (Ter Braak, 1988) on one half of the data set, field pH may be inferred from the species data, showing the indicator value of the community structure. In all analyses it was found that the pooled p/a data gave essentially the same result as the relative abundance data.

The effects of changes in the pH and Aluminium content of water on the growth rate of selected species of green algae was investigated using a laboratory-scale recirculating miniature artificial stream apparatus which allowed six variations to be tested at one time. Algae were grown attached to microscope slides. Growth rates were measured by counting cells in individual filaments over several successive time intervals. Mixed cultures of up to eight species were employed.

Species characteristic of the lower-pH streams such as *Hormidium subtile*, *Geminella* 8µm., *Stigeoclonium* 5µm. and some species of *Mougeotia* have a pH optimum for growth rate between pH 4.5 and 5.5. Species characteristic of circumneutral streams, *Draparnaldia sp. Stigeoclonium* 8µm. and *Oedogonium* species, have a pH optimum between pH 5.0 and 6.0 or above. *Hormidium subtile* and *Geminella* 8µm. can grow at a reduced rate in a monomeric Aluminium concentration of 200 µg l^{-1} in which the majority of species tested are rapidly killed. Evidence was found for ecotypes with respect to pH in the genus *Stigeoclonium*.

Biomass in natural waters was positively correlated with pH in contrast to some previous reports. In mixed cultures in the laboratory, the maximum biomass developed was in the range pH 4.5 to 5.5, around the pH optimum of the species present. At higher pH values (5.5 to 6.0) diatoms were predominant giving a brown-coloured periphyton layer which is less visually obtrusive than the bright green growth of the filamentous chlorophytes. Therefore anecdotal reports of an increased biomass upon acidification may reflect only a shift to more visible species. Inhibition of diatom growth by Germanium addition provided no evidence in favour of competition between diatoms and chlorophytes.

Differences in community structure and changes in biomass with pH in laboratory culture cannot be ascribed to changes in invertebrate grazing or heterotrophic microbial activity. It is concluded therefore that differences between species in tolerance towards pH or associated water chemistry variables are sufficient to explain differences in algal community structure in the field.

1. INTRODUCTION

A trend towards the acidification of fresh waters has been noted in several regions of the world over the last 20 years. This process is popularly ascribed to the effects of 'Acid Rain', but in reality acid precipitation is only one of a number of factors which predispose a catchment to acidifcation of its drainage water (Seip and Tollan, 1978).

The rainfall may be acidified beyond its natural acidity (due to dissolved CO_2) (Likens and Bormann, 1974) by SO_2 and NO_x derived both from natural sources and from the combustion of fossil fuels. In addition, particulate pollution from this latter source, 'dry deposition', can contribute acidity (Irwin, 1985). Salts in rainfall derived from sea-spray also contribute ultimately to the acidification of drainage water (Seip, 1980; Krug *et al*, 1985).

Catchments vary in their susceptibility to acid deposition. Soils, both mineral and organic, have the capacity to neutralize acidity (Bache, 1984; Edmunds and Coe, 1986). Catchments with deep soils, especially of a neutral or alkaline nature, and with slight slope, allowing slow drainage, effectively neutralize precipitation (Bache, 1984; Edmunds and Coe, 1986; Hornung *et al*, 1986). On the other hand catchments in upland areas with steep slopes, with exposed, slowly weathering bedrock and thin mainly peaty soils, are very susceptible to acidification (Bache, 1984).

Vegetation also influences soil acidity, and hence the pH of water passing through the soil. Trees may alter the precipitation pattern in a catchment and may, in addition, trap dry deposition (Mayer and Ulrich, 1974; Mayer, 1983). The growth of vegetation involves the uptake of nutrient ions from the soil, resulting in a net increase in soil acidity (Nilsson *et al*, 1982; Edmunds and Kinniburgh, 1986) the extent of which differs with species (Henderson *et al*, 1977; Nilsson *et al*, 1982; Skeffington, 1983; Matzner and Ulrich, 1983; Hornung, 1985). This process may be reversed when the vegetation dies back, or when a forest is cleared if the brushwood is left to decompose (Krug and Frink, 1983). However some plants such as ling (*Calluna vulgaris*) contain phenolic compounds which inhibit decomposition to such an extent that nutrients remain trapped in a layer of organic material, eventually forming acidic peat (Rosenqvist *et al*, 1980).

The rate of drainage through the soil also influences the acidity of drainage water. Prior to the planting of trees in the past it has been the practice to plough and drain hill slopes, reducing the residence time of water in the soil. Channeling of flow through passages provided by tree roots may also occur (Bache, 1984). In addition, the drying out of previously saturated organic soils may cause acidification by the oxidation of sulphides (Van Dam, 1988).

In certain areas of the world sufficient of these predisposing conditions have been present to lead to the acidification of fresh waters. This has been reported from areas of the Canadian Shield, Nova Scotia and the Adirondacks in North America (Davis *et al*, 1978; Harvey, 1980; Krug *et al*, 1985), large areas of Scandinavia (Seip and Tollan, 1978), and Wales (Stoner *et al*, 1984), Galloway (Wright *et al*, 1980) and Western Scotland (Harriman and Morrison, 1982; Harriman and Wells, 1985) in Britain.

The most economically serious consequences of acidification are the deleterious effects on stocks of salmonid fish indigenous to the nutrient-poor and highly oxygenated waters found in these upland areas. The effects may be due to low pH alone or in combination with associated low Ca^{2+} and high Al concentrations. Invertebrates which form an important food source for these fish are also adversely affected (Kinsman, 1984; Burton *et al*, 1985; Kullberg and Petersen, 1987).

It has been suggested that microbial degradation of organic material in fresh water is reduced by acidification (Hendrey and Wright, 1975; Traaen, 1980; Allard and Moreau, 1985); more precisely, bacterial decomposers being progressively replaced by fungi which have a lower rate of mineralization (Overrein *et al*, 1980). However this has been contested more recently (Arnold *et al*, 1981).

Acidified streams and lakes are susceptible to 'blooms' of attached green algae when compared with less acid streams (Hendrey, 1976; Kinsman, 1984; Stokes, 1981, 1986; Turner *et al*, 1987) which replace diatoms as a dominant group.

In the water column of lakes species changes occur among the phytoplankton, although there may not be any change in biomass (Molot *et al*, 1990; Raddum *et al*, 1980). There is a shift towards acidobiontic species of diatoms and chrysophytes, which have been utilized as indicators of lake pH, allowing palaeoecological surveys of lake acidication to be carried out from sediment cores (Batterbee, 1984; Charles, 1985; Charles and Smol, 1988; Davis and Berge, 1980; Dixit and Dickman, 1986; Flower and Batterbee, 1983; Holmes *et al*, 1989; Van Dam *et al*, 1980; Van Dam, 1988).

Acidification induced changes in periphyton composition and biomass in streams and the littoral zone of lakes are highly visible and may act as an early indicator of acidification (Turner *et al*, 1987) but there has been speculation as to the precise cause of biomass changes (Hall *et al*, 1980; Stokes, 1981, 1986).

The possibilities are:

(a) a direct effect of pH favouring acidobiontic species;
(b) removal of grazing organisms allowing unconstrained accumulation of particular species;
(c) a decrease in the rate of decomposition of inactive algal biomass;
(d) a decrease in competition from species which are inhibited by low pH (Stokes, 1986).

Algae form at least part of the diet of aquatic invertebrates (Eichenberger and Schlatter, 1978; Fulton, 1988; Hart, 1985; Hill and Knight, 1987, 1988; Jacoby, 1987; Johnson, 1987; McAuliffe, 1984; Mason and Bryant, 1975; Peterson, 1987; Slack, 1936; Titmus and Badcock, 1981; Yoshitake and Fukushima, 1985).

The abundance of invertebrate grazers in acidified streams is reduced (Arnold *et al*, 1981) and an inverse relationship between algal biomass and grazer abundance has been noted in experimentally manipulated systems including artificial acidification, invertebrate enhancement or exclusion by physical means or insecticide treatment (Eichenberger and Schlatter, 1978; McAuliffe, 1984; Yasuno *et al*, 1985; Jacoby, 1987; Feminella, 1989; Winterbourn, 1990) as well as by natural processes other than acidification (Lamberti and Resh, 1985b).

Selective grazing has been found to influence algal species composition (De Nicola *et al*, 1990; Hart, 1985; Hill and Knight, 1987; 1988; Jacoby, 1987; Pringle, 1985). Conversely, the quality of food represented by periphyton may limit the density of grazing invertebrates (Collier and Winterbourn, 1987) and invertebrates may be indirectly affected by pH decrease via consequent changes in decomposition rates (Hildrew *et al*, 1984).

Hendrey (1976) found that while algal cell numbers and biomass were higher at pH 4 than pH 6, the rate of carbon fixation was decreased, suggesting that the reason for biomass accrual might be reduced heterotrophic activity. There appears to be as yet no evidence concerning competitive interaction between algal species.

A direct favouring of acidobiontic species is implicit in the use of diatoms and chrysophytes as biological indicators. However for the most part the indices used are based solely on correlations between species abundance and environmental factors in field studies, and have not been checked experimentally (Gensemer, 1990a).

Possible routes for pH to influence algal species composition and productivity are numerous. There may be a direct effect of pH *per se* on the metabolic processes, with different species responding differently either in their ability to exclude hydrogen ions or through some acidobiontic species having metabolic processes adapted to low internal pH (Lane and Burris, 1981).

Correlations between species abundance and pH may be due to concomitant effects upon metal concentrations, nutrients or light penetration.

Many metals are mobilized from sediments and catchments by low pH. The most important in terms of its abundance and effects is aluminium, which is known to be toxic to fish (Muniz and Lievestad, 1980; Grahn, 1980; Robinson and Deano, 1986; Ormerod *et al*, 1987; Birchall *et al*, 1989; Dietrich and Schlatter, 1989; Holtze and Hutchinson, 1989), and to increase drift of macroinvertebrates (Weatherley *et al*, 1988). Heavy metals may also be solubilized and may reach toxic levels under particular local circumstances, as in the case of acidic mine drainage (Tease and Coler, 1984; Deniseger *et al*, 1986).

Toxicity to algae of aluminium (Helliwell *et al*, 1983; Campbell and Stokes, 1985; Folsom *et al*, 1986; Claesson and Tornqvist, 1989; Pillsbury and Kingston, 1990) and other metals (McLean, 1974; Harding and Whitton, 1976; Say *et al*, 1977 a,b; Marshall and Mellinger, 1980; Say and Whitton, 1980; Leland and Carter, 1984; Peterson *et al*, 1984; Campbell and Stokes, 1985; Deniseger *et al*, 1986; Starodub *et al*, 1987; Luderitz and Nicklisch, 1989) has been demonstrated.

In the case of aluminium, effects on standing crops and possibly also on species composition of algae may be indirect due to complexation reactions. Aluminium may complex with phosphorus to form insoluble $Al_3 PO_4$ precipitates, effectively reducing phosphorus availability (Minzoni, 1984; Nalewajko and Paul, 1985). It also complexes with silicon, which is normally present in much higher concentrations than is phosphate, especially at higher pH. This may protect fish against the toxic effects of Al (Birchall *et al*, 1989) and will presumably have an ameliorating effect on other members of the biotic community.

Since the maximum solubility of Al is around pH 5.0 (Nordstrom and Ball, 1986), silicon complexation will be greatest around this pH or higher. However the toxicity of Al depends upon the molecular species, monomeric aluminium being the most toxic and polymeric or complexed Al the least toxic (Helliwell *et al*, 1983; Miller and Andelman, 1987; Steinberg and Kuhnel, 1987; Gjessing *et al*, 1989; Tipping *et al*, 1989).

Monomeric Al is most abundant at low pH in the absence of complexing molecules, principally phosphorus, silicon and humic acids. At low pH in many catchments with a dense development of peat in the organic A horizon, the drainage waters are stained brown with organic acids (humic and fulvic acids). These have a high ion exchange capacity and may complex with Al, releasing H^+ ions and forming a precipitate. Thus humics may act as buffers in natural waters (Henriksen *et al*, 1989; Johannessen, 1980) unless high levels of Al are released from the mineral layers of the catchment (Turner *et al*, 1985), when they will acidify the water but protect against the deleterious effects of Al (Robinson and Deano, 1986). On the other hand increased H^+ may reduce the toxicity of some metals by increasing competition for binding sites (Campbell and Stokes, 1985). Other metals may in fact be micronutrients and an increase in their availability may enhance algal growth (Eichenberger, 1979). Humic material can however also complex these metal ions and thus sequester essential minerals so that it shows inhibitory effects on algal growth (Jackson and Hecky, 1980). Phenolic compounds may inhibit microbial metabolism (Freeman *et al*, 1990).

The intense colour imparted by humics also reduces light penetration in lakes and may reduce the euphotic zone effectively limiting productivity. One consequence of acidification involving increased aluminium leaching from the catchment is an increased clarity of lakes (Shearer *et al*, 1989; O'Grady and Brown, 1989; which may account for some of the changes in algal composition and density observed (Turner *et al*, 1987).

Changes in the pH of water also influence the availability of inorganic carbon to aquatic plants. At high pH the buffering is provided by carbonate/bicarbonate and plants take up carbon for photosynthetic fixation as HCO_3^-. The source is large and therefore not limiting at pH values greater than 6, but below this HCO_3^- diminishes in importance and is replaced by dissolved CO_2 (Turner *et al*, 1987). The concentration of CO_2 in solution is limited by its rate of diffusion from the atmosphere and its solubility, which depends on temperature, pH and other solutes. Thus it may be supposed that acidification might lead to dissolved inorganic carbon (DIC) limitation under conditions of light and nutrient sufficiency for periphyton and phytoplankton growth. Enhanced growth of periphyton in the riffle zones of acidified streams could reflect enhanced availability of dissolved CO_2 because of turbulent mixing at the air-water interface.

The shift from HCO_3^- to CO_2 based metabolism may be a major factor influencing the composition of algal communities, as some species are able to utilize only one of these sources (Raven and Beardall, 1981; Turner *et al*, 1987).

The interrelationship of the different biotic components of the ecosystem complicates any attempt to understand the effects of environmental factors such as increasing acidification.

Salmonid fish feed on aquatic invertebrates and terrestrial invertebrates which fall into the water. The invertebrates are carnivores, algal grazers, detritus or filter feeders, utilising native or allochthonous material.

In this investigation an assessment has been made of the influence of pH and related chemical variables on total algal standing crop and species composition in the field, and on growth rates of individual species in the laboratory.

The Trossachs area of the Scottish Highlands is one of the areas seriously affected by acidification (Harriman and Morrison, 1982). Monitoring of water chemistry, fish and invertebrate populations, has been carried out by the Scottish Office Agriculture and Fisheries Department (SOAFD), Freshwater Fisheries Laboratory, Pitlochry, since 1975. The Loch Ard catchment area contains a variety of streams spanning a range of mean pH values between approximately 4.0 and 7.0. This is due to the fact that the area straddles the Highland Boundary Fault, with slowly weathering schists to the north-west and more easily weathering, thus 'more basic', rocks to the south-east of the fault line. Superimposed on this is a range of degree and age of afforestation. Mean stream pH is inversely related to the age of trees in the catchment (Harriman and Morrison, 1982; Ormerod *et al*, 1989), so that streams in the Loch Ard area may be expected to differ in pH in both space and time.

A preliminary survey of benthic algae in the Loch Ard catchment was carried out in 1985 (Kinross, 1985). This indicated that differences in species distribution existed between streams of differing mean pH. The present work was carried out in order to refine these observations and relate the distribution of species with their responses to experimental manipulation of the growth conditions.

2. MATERIALS AND METHODS

2.1 CHOICE OF SITES

Sampling sites on each burn were chosen on the basis of both accessibility and suitability. In some cases reasonably open sites with good light availability are rare due to close planting of forestry, and the only open sites occur where the burn crosses a fire-break, pylon line or the line of the Loch Katrine aqueduct. The locations of the burns and sampling sites are indicated in Fig. 1.

Most sampling sites are near the staff gauges installed by the SOAFD Freshwater Fisheries Laboratory; these are situated on straight, level reaches as far as possible, which thus also normally provide good sites for the location of the artificial substrates. The Freshwater Fisheries Laboratory has also situated its composite water samplers (sampling over a 1 week period) at these points. Additional sites were chosen upstream on some burns (see Fig. 1).

Characteristics of the burns and sites are shown in Table 1.

2.2 FIELD SAMPLING

Sampling was carried out in the Loch Ard area on a regular basis. Initially sampling was carried out every two weeks, but later approximately monthly. Samples of periphytic algae and stream water were collected and measurements made of temperature, staff gauge reading, and integrated incident light.

2.2.1 Sampling methods for algae

2.2.1.1 Natural Substrates

Filamentous samples were taken by hand, either picked with fingers or as a fingernail scraping. Diatoms and other small, closely adhering periphyton were taken by brushing stones vigorously with a 25mm paintbrush and rinsing into a plastic tub. The brush was also used for taking filamentous samples underwater against the current.

For biomass estimation, stones were removed from the streams, put in labelled polythene bags and transported to the laboratory. Stones were selected on the basis of size and shape in order to make extraction of pigments possible, and one was generally taken from both a region of fast and slow current.

FIGURE 1 LOCATION OF SAMPLING SITES IN THE LOCH ARD AREA

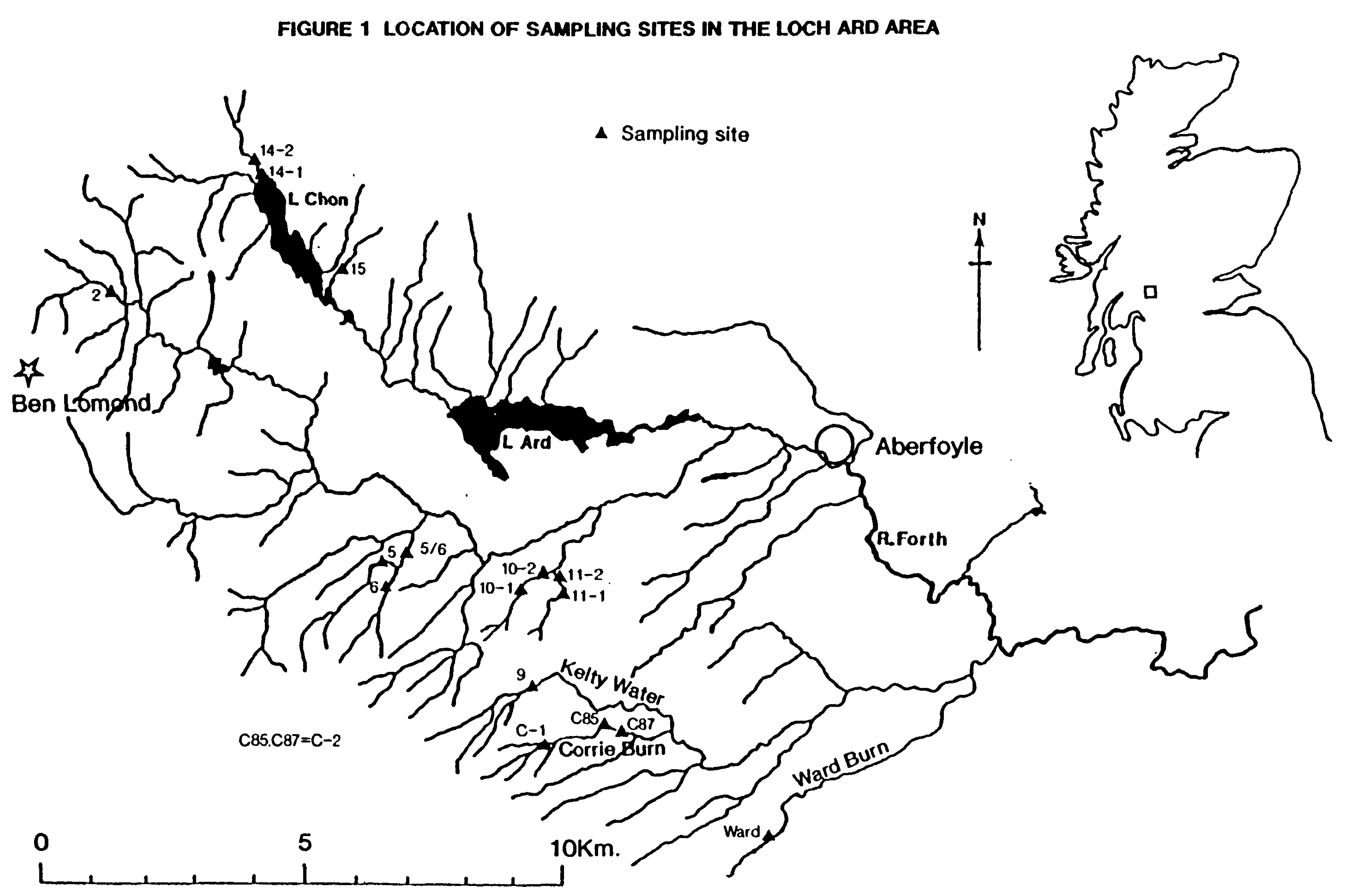


TABLE 1
PHYSICAL CHARACTERISTICS OF SITES

No.	1 National Grid Reference	2 Alt. m.	3 Catchment Area Km²	4 % Forest	5 Age Forest Years (1986)	6 Stream Order[1]	7 Mean Gradient	8 Width m.	9 Bed Stability	10 Silting	11 Substrate Nature Size	12 Direction of Course	13 Bankside Vegetation	14 Light Regime	15 PAR % B.6	16 Estimated PAR % (For CANOCO)
SITE																
14-1	NN 413 071	110	2.70	80	31	2	0.079	2.0	1.0	-	Cobble, Stones	W - E	Conifer, Willow, Grass	Some Shade	61	
14-2	NN 412 073	120	2.00	80	31	2	0.075	2.5	2.0	-	Bedrock	W - E	Bog Myrtle, Willow	Slight Shade	-	90
15	NN 430 046	110	0.70	50	5	2	0.262	2.0	1.0	-	Cobble/Boulder	N - S	Alder	Much Shade	-	40
2	NN 388 043	150	5.25	0	-	3	0.210	3.0	0.0	+	Cobble, Boulder	SW - NE	Grass, Bracken	Open	80	
5	NS 438 992	140	1.56	55	8	2	0.153	2.0	0.0	+	Slate Pebble	SW- NE	Grass	Open: High Banks	78	
6	NS 438 989	150	1.14	45	6	3	0.176	2.0	1.0	-	Sl. Pebble/Cobble	SW - NE	Grass/Few Alders	Open	100	
5+6	NS 440 992	130	2.70	50	7	3	0.151	3.0	2.0	-	Cobble/Boulder	SW - NE	Alder	Slight Shade	-	70
9	NS 446 967	170	2.10	55	37	2	0.104	2.0	0.0	+	Cobble/Pebble	S - N	Grass, Birch	High Banks	73	
10-1	NS 465 987	110	0.73	96	33	2	0.048	1.0	0.5	+	Slate Pebble	S-N	Conifers	Much Shade	42	
10-2	NS 469 988	110	0.93	*96	33	2	0.047	1.0	0.5		Pebble/Stone	S-N	Conifers, Grass	Some Shade from South	-	90
11-1	NS 472 985	140	1.47	91	33	2	0.034	1.0	2.0	-	Stone/Gravel	S - N	Conifers, Grass, Bog Myrtle	Slight Shade from South	69	
11-2	NS 470 988	110	1.57	91	33	2	0.053	3.0	2.0	-	Cobble/Gravel	E - W	Conifers, Birch	Much Shade from South	-	50
C-1	NS 465 959	170	1.24	84	38	2	0.148	3.0	0.0	+	Gravel/Cobble/ Boulder	SE - NW	Conifers	Much Shade	-	40
C 2	NS 482 958	110	2 64	#84	38	2	0.133	4.0	1.0	+	Gravel/Cobble/ Boulder	SW-NE	Conifers, Alder	Slight Shade	57	
Ward	NS 509 936	90	1.50	90·	10·	1	0.061	0.75	0.0	+	Gravel/Cobble	S - N	Grass	Open	-	90

*Felled in 1987
#1987 W. bank felled
·Estimates

[1] Ref. Shreve, 1956

0 = unstable
1 = moderate
2 = stable

Gravel < 5 cm
Pebble > 5 cm
Stone > 10 cm
Cobble >15 cm
Boulder > 20 cm

2.2.1.2 Artificial Substrates

i. 15 cm square white glazed ceramic tiles were mounted in groups of three in Dexion frames using 7mm plastic channel. The frames were securely anchored in the stream bed with Dexion stakes driven 0.5-1.0m into the bed. Tiles were sampled by removing the tile and either brushing off any growth from the glazed surface into a plastic bottle, or transporting the entire tile back to the laboratory for chlorophyll extraction.

ii. Glass microscope slides (76 x 26 mm) were mounted in pairs back-to-back in Perspex racks, holding 6 pairs in total. The racks were fastened to a building brick with silicone rubber cement and plastic-coated tying wire. Bricks were frequently dislodged in spates and the slides lost, so they were additionally anchored to the tile racks with tying wire. A Perspex strip held down with nylon 4BA screws retained the slides. Slides were removed from the racks and transported to the laboratory in polythene slide holders (Luckhams) full of stream water.

2.2.2 Transport of Samples

All field samples were transported to the laboratory packed in ice, and were kept on ice until examined or extracted for pigment analysis, as appropriate.

2.2.3 Water Sampling

Samples of streamwater were taken at the sampling site in acid-washed 250ml polyethylene bottles which had been rinsed out with distilled water and again rinsed three times in the stream water before being filled. During warm weather the samples were kept in an ice-box until being returned to the laboratory.

2.2.4 Temperature

Temperature was measured at each sampling site with a mercury-in-glass thermometer. In addition at certain sites a maximum-minimum thermometer was encased in a steel mesh sheath and attached to the tile rack. These were cheap, crude instruments so their calibration was checked in the laboratory and appropriate corrections applied as necessary.

2.2.5 Flow

Staff gauges to measure the depth of water at relatively level reaches of the burns have been installed by the Freshwater Fisheries Laboratory at some sites. In addition the Forth River Purification Board had recently installed flumes on burns 9,10 and 11, with staff gauges alongside. These were read at the time of sampling. To obtain a value for volumetric flow a calculation based on mean width and bottom characteristics must be made. This has not been done as the total volume of flow gives no direct indication of velocity at specific points. The staff gauge readings are thus used for within-stream comparisons only.

2.2.6 Light

Photosynthetically Active Radiation (PAR) was measured at selected sites, in rotation, by comparison with an 'open' site. The instruments used were home-made electronic integrators, and are fully described in Appendix 2. The sensors were designed to have a spectral response similar to the absorption pattern of some of the common filamentous algal species. Instantaneous measurements of PAR could be made with one of these sensors in conjunction with a modified voltmeter.

On site, the sensors were bolted to Dexion stakes in the stream bed, or to the tile racks. The top of the sensor was levelled with a spirit level, and located approximately 5 cm above 'normal' water level. The sensors were frequently submerged during floods and proved to be waterproof, but drifting vegetation occasionally interfered with a reading, which had to be discounted. Readings were a measure of integrated PAR between visits. Corrections were applied to readings to take account of different times of reading between integrators, on the basis of a count-rate determined at time of sampling. A further correction was necessary to relate the counts from each integrator to the reference instrument; these corrections were determined beforehand by simultaneous comparison of all instruments.

The results are expressed as a percentage of the PAR incident at the reference 'open' site at Burn 6. These are taken as the basis for comparing the light regimes at different sites. A conversion of the count of each instrument into PAR (μmoles m^{-2} s^{-1}) was also obtained by comparison with a direct reading meter (see Appendix 2).

2.3 LABORATORY METHODS

2.3.1 Microscopic examination of algal samples

Aliquots of each sample were removed and mounted under coverslips.
Samples were examined live, without staining, using a Leitz Ortholux microscope equipped with phase-contrast.
Cell dimensions were calculated by measurement against an eyepiece graticule. All measurements of size were carried out with a x40 objective, x12.5 eyepiece, total magnification x500.

2.3.2 Photography

Photographs were taken using a Leica 35mm camera back on the Leitz microscope. Kodak Ektachrome 200 ASA slide film was routinely used, processed by the Napier Polytechnic Photography Department.
A light blue filter was used to compensate for the colour of the tungsten light. The supply current to the lamp was kept constant to give consistent colour rendition.

2.3.3 Species identifications

The keys in Prescott (1970) and Starmach (1972) were used to identify algae to the genus level. Other keys used were in Randhawa (1959) and Ramanathan (1962).
The criteria of Cox and Bold (1966) were applied to the identification of *Stigeoclonium* species.

2.3.4 Species abundance

During examination of samples, the relative abundance in each sample of different taxa was estimated following microscopic screening of the whole aliquot on the slide. Several aliquots were examined if the sample appeared heterogeneous.
The estimates of abundance rank taxa in 7 categories. These are abundant; abundant/common; common; common/frequent; frequent; frequent/rare; and rare. A species is recorded as abundant if it appears to constitute more than approximately 30% of the total algal volume present, and rare if it is present only once to a few times in the whole sub-sample.
Between these categories there is obvious opportunity for uncertainty in the ranking of individual taxa due to the subjective nature of the ranking process, so the rank accorded may have an error of plus or minus one category.

2.4 TREATMENT OF SAMPLES

2.4.1 Biomass Estimation

Two methods were used for biomass estimation. These were Pigment Extraction and Ash-Free Dry Weight (AFDW). Where possible both pigment extraction and Ash-Free Dry Weight analysis were carried out sequentially.
Brushed samples from tiles were divided into aliquots, following sonication if necessary with very concentrated filamentous samples. Replicate aliquots were filtered onto Whatman glass fibre filter papers (type GF/C), an aliquot retained fresh for microscopic examination, and a further aliquot preserved in 6-3-1 (water : 95% alcohol : formaldehyde) preservative (Prescott, 1970).

2.4.1.1 Pigment extraction

For chlorophyll analysis, extraction was carried out in 90% acetone saturated with $MgCO_3$, overnight in a refrigerator. For carotenoid and chlorophyll analysis, $MgCO_3$-saturated 90% methanol, at 55° for 1 hour (Foy, 1987) was used.
Filters were frozen at -20°C during the earlier part of the investigation, but this was discontinued when it was found that some species give incomplete extraction following freezing.
The extractant was changed to 90% methanol after it was found that some unialgal cultures gave very poor extraction in acetone (see Section 4.3).
Filters were immersed in the extractant without maceration (frozen filters were torn up and placed in the extractant). The contents of the tube were mixed by periodic inversion.
Slides were extracted by draining them briefly and then immersing in extractant in the Luckham's slide containers.
Extraction of intact tiles and stones was carried out by placing them upside-down in a polythene sandwich box or similar container with a tight-fitting lid. Allowance was made for the volume of water contained in the algal biomass by including a volume of 100% extractant equivalent to ten times the estimated water volume, along with sufficient 90% acetone (or methanol) saturated in $MgCO_3$ to wet the top (i.e. formerly exposed) surface of the tile or stone.

2.4.1.2 Ash-Free Dry Weight Determination

Following extraction, filters and slides were removed from the containers and dried in an oven at approximately 60°C to constant weight. They were then subjected to incineration for 1 hour at 550°C in a muffle furnace before being re-weighed on a Stanton balance to determine weight loss on ignition (AFDW). Each weighing is estimated to be accurate to ±0.1 mg. To prevent loss of loose material, filters and slides were weighed wrapped in Aluminium foil. Al melts at about 660°C and no loss of weight from Al foil at 550°C was found. GF/C filters (47mm diam.) lose approximately 0.7mg on incineration at 550°C. They were not pre-ashed as this makes them brittle so they break up during filtration or pigment extraction. A subtraction of 0.7mg was made to accommodate this weight loss. Inaccuracy is estimated as ±0.2 mg. Slides treated in this way can then be mounted in Naphrax (Northern Biological Supplies) for diatom examination.

2.4.1.3 Chlorophyll analysis in pigment extracts.

Extracts were centrifuged in stoppered tubes at 3,000 rpm for 20 minutes. Chlorophyll-a was determined by the trichromatic method of Parsons and Strickland (1963).

Absorbance was measured at 750, 663, 645 and 630nm in 4cm glass cells against a 90% acetone or methanol blank. For the analysis of phaeophytin, acetone extracts were then acidified in the cell with 8 drops of 0.1N HCl and A_{750} re-read. After1-2 minutes the A_{665} was read. These readings enable phaeophytin-a and chlorophyll-a to be distinguished (Lorenzen, 1967).

Pigment yields per extract (slide or filter) were calculated from the following equations:

<u>Trichromatic</u>

$$\text{chl-a}(\mu g) = \frac{11.64\,(A_{663}-A_{750}) + 2.16\,(A_{645}-A_{750}) - 0.1\,(A_{630}-A_{750}) \;\times\; \text{Extract Volume, ml.}}{4\text{ (path length) cm}}$$

(SCOR/UNESCO 1966)

<u>For Phaeophytin</u>

$$\text{chl-a}(\mu g) = \frac{26.73\,[(A_{663}-A_{750}) - (A_{665}-A_{750})] \times \text{Extract Volume, ml}}{4}$$

$$\text{phaeo-a}(\mu g) = \frac{26.73\,[1.7\,(A_{665}-A_{750}) - (A_{663}-A_{750})] \times \text{Extract Volume, ml.}}{4}$$

(Lorenzen, 1967)

Variations in the formulae necessary when using methanol are dealt with in Section 4.3 of the Discussion.

[In methanol the absorbance maximum is at 664 nm and is not shifted following the conversion chlorophyll-a to phaeophytin-a. However the extract must either be acidified and then neutralized, or transferred to 90% acetone for reading (Marker *et al*, 1980).]

2.4.1.4 Carotenoid analysis

Carotenoid is determined as 'microscopic pigment units' (μspu) from methanol extracts (Foy, 1987). It is calculated using an extinction coefficient of 4 (Strickland *et al* , 1968).

$$\text{Carotenoid} = \frac{4(A_{480}-A_{750}) \times \text{Extract volume, ml}}{4\ (\text{path length, cm})} \quad \mu\text{spu}$$

2.4.2 Surface area of substrates

Tiles measured 15 x 15cm, giving a total surface area of 225cm^2. A strip measuring approximately 3mm wide was not exposed up each side due to the plastic channel; this was compensated by the exposed vertical surfaces at the leading and trailing edges. Similarly the surface area of slide pairs was calculated as 20cm^2, including the sides of the slide. The surface area of stones was estimated by wrapping the exposed upper surface with aluminium foil and estimating the area from the weight of foil (Naiman, 1983).

2.5 ANALYSIS OF WATER SAMPLES

2.5.1. pH

pH was measured to 0.01 pH unit with an Orion model 611 pH meter equipped with a Ross combination electrode. The filling solution (3M KCl) was replaced before reading a set of field samples. The pH was read in a stirred sample. When a steady reading was obtained the sample was discarded and a fresh portion added, and the pH reading was taken as soon as it stabilized. The stirrer was then stopped and a reading taken of the unstirred sample. The samples were read at a temperature near to the field temperature at the time of sampling.

2.5.2 Absorption (optical density) measurements

The absorption of water samples was read at 400, 350 and 250nm in quartz cuvettes in a Unicam SP8-100 recording spectrophotometer. This instrument was also used to obtain scans of chlorophyll extracts and cell suspensions, used in the design of the PAR sensors. Absorption in the U.V. band is highly correlated with total organic carbon concentration of water.

2.5.3 Chemical analyses

The following analyses were employed on field samples, and on synthetic stream water during growth experiments.

Calcium was determined by AAS in a nitrous oxide-air flame in a Perkin-Elmer Model 373 Atomic Absorbance Spectrophotometer. Phosphate was determined by the stannous chloride method (APHA, 1985). Nitrate was determined by conversion to nitrite by the spongy cadmium method, followed by assay of nitrite by reaction with sulphanilamide (Elliott and Porter, 1971). This results in an estimate of Total Oxidised Nitrogen (TON). Aluminium was determined by the catechol violet method (Seip *et al*, 1984; Rogeberg and Henriksen, 1985). Fractionation into labile and non-labile Al is carried out by passage over ion exchange resin (Driscoll, 1984). Silicon was determined by the oxalic acid-molybdate method (Parsons *et al*, 1984). In addition the SOAFD Freshwater Fisheries Laboratory routinely monitors a range of determinands in one-week composite samples. The full range of determinations includes pH, conductivity, Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Al, Fe, Si, SO_4^{2-}, Cl^-, PO_4^{3-}, and NO_3^-, but is not carried out on all samples.

2.6 ALGAL GROWTH EXPERIMENTS

2.6.1 Media

Initial growth experiments were carried out in Chu no. 10 medium (Nichols, 1973) and attempts to identify *Stigeoclonium* species employed medium $BBMPTB_{12}$ (Cox and Bold, 1966).

For measurements of growth rates and experiments to determine changes in species composition, two new media were formulated based on chemical analysis of two of the Loch Ard streams (data from SOAFD, Pitlochry). Mean values over several years were used; with more frequent sampling in recent years the means may be biassed towards recent values.

Media were formulated using only the ions Na^+, K^+ Mg^{2+}, Ca^{2+}, Al, Fe, Si, SO_4^{2-}, Cl^- H^+, PO_4^{3-}, NO_3, and Mn^{2+}, slight discrepancies in the ion balance being attributed to organic acids (Cronan *et al*, 1978; Krug *et al*, 1985). No attempt was made to accommodate these in the media.

The recipes for media 'B9' and 'Corrie' are given in Table 2, and the concentrations of ions are compared with $BBMPTB_{12}$ in Table 3.

Media are made up as concentrates (100 times) and diluted for use in glass-distilled water. Both media, especially 'Corrie', contain precipitates and have to be thoroughly resuspended before aliquots are removed for dilution.

Manipulation of aluminium and silicate concentrations was carried out in Corrie medium. The amount of sodium silicate solution was reduced from 0.93 ml to 0.079ml per litre of concentrate, a decrease from 47 to 4 μM, to constitute treatment B. Treatment C had the total aluminium target level increased from 40 to 146 $\mu g l^{-1}$ (the same concentration as in B9 medium), although analysis showed that a higher final concentration was achieved in practice. Treatment A was unmodified Corrie medium. The pH of A,B and C was maintained at 5.5, while treatment D was unmodified B9 medium at its 'natural' pH of 4.6.

TABLE 2
RECIPES FOR SYNTHETIC STREAMWATER MEDIA BASED ON L. ARD BURNS

Medium B9	100x concentrate per litre:
Ca $(NO_3)_2 4H_2O$	94mg
NaH_2PO_4	180mg
$MgSO_4.7H_2O$	715mg
$CaCl_2.6H_2O$	416mg
$FeSO_4.7H_2O$	200mg
$AlK(SO_4)_2.12H_2O$	260mg
Na_2SO_4	780mg
$MnSO_4.4H_2O$	45mg
*sodium silicate solution	0.53 ml
1N.HCl	2.8ml

pH of 1x is ~ 4.6

Medium 'Corrie'	100x concentrate per litre:
Ca $(NO_3)_2 4H_2O$	354mg
NaH_2PO_4	24mg
$MgSO_4.7H_2O$	1970mg
$CaCO_3$.	800mg
KCl	37mg
$FeSO_4.7H_2O$	71mg
$AlK(SO_4)_2.12H_2O$	71mg
Na_2SO_3	110mg
Na_2SO_4	750mg
NaCl	41mg
*Sodium silicate solution	0.93ml

pH of working-strength dilution may rise to > 7.0 on prolonged stirring

*Contains approximately 30% SiO_2 and 12% Na_2O w/v

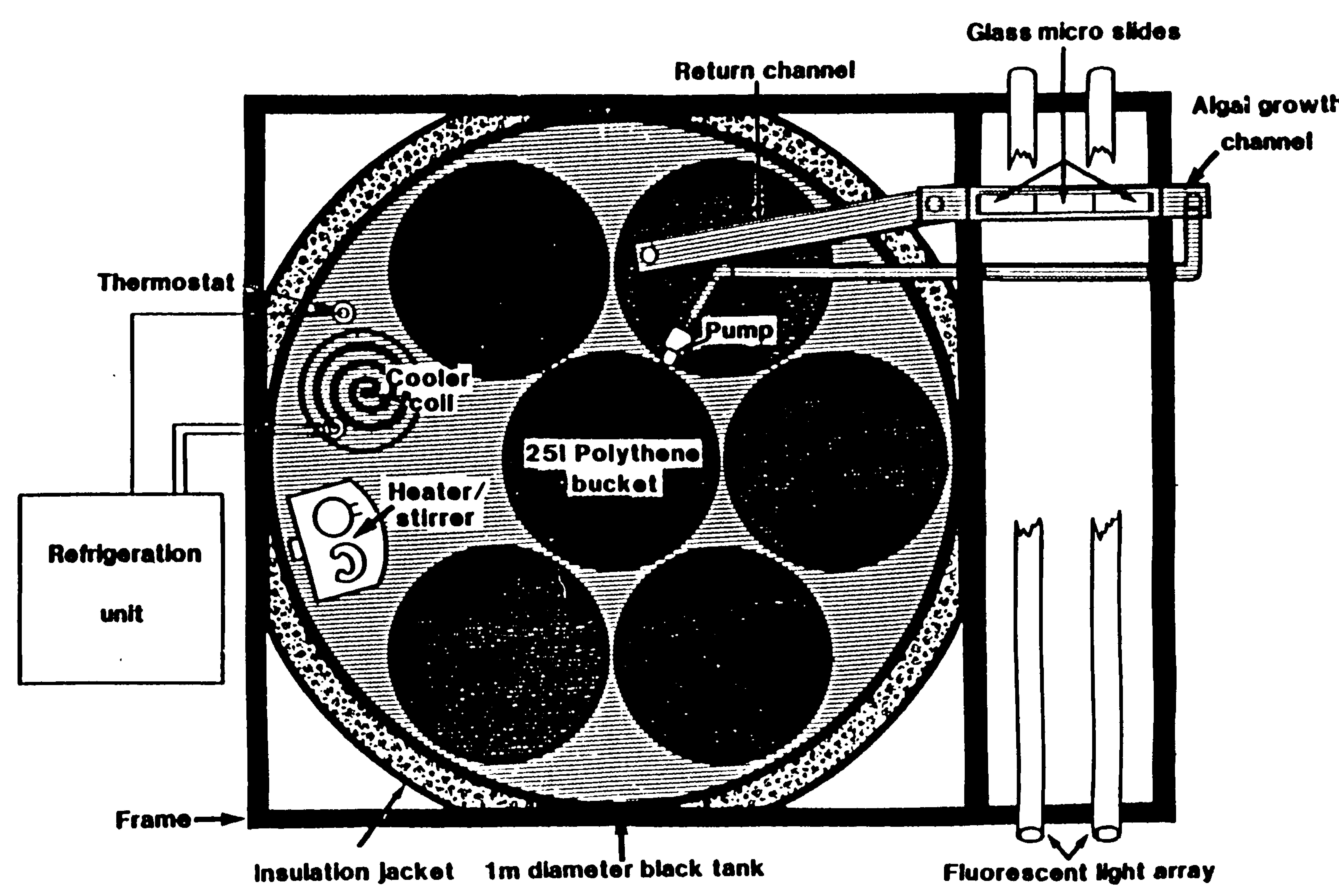
TABLE 3

COMPARISON OF ARTIFICIAL STREAMWATER MEDIA USED IN GROWTH RATE AND TAXONOMIC STUDIES

	B9		CORRIE		$BBMPTB_{12}$	
	$\mu eq\ l^{-1}$	$mg\ l^{-1}$	$\mu eq\ l^{-1}$	$mg\ l^{-1}$	$\mu eq\ l^{-1}$	$mg l^{-1}$
Al	16	0.146	4.4	0 .040		
B					368	2.0
Ca	46	0.92	190	3.8	400	8.0
Co					3.4	0.2
Cu					12	0.76
Fe		0.40		0.19	36	1.0
K	5	0.195	7	0.273	2750	107.3
Mg	57	0.684	163	1.956	600	7.2
Mn	100	2.75				
Mo					15	0.5
Na	144	3.31	175	4.025	3400	78.2
Si		1.6		2.8		
Zn					62	2.0
CO_3			180	5.4		
NO_3	8	0.112*	30	0.42*	3000	42*
PO_4	45	0.47*	5	0.05*	48	49.6*
SO_4	299	4.8*	193	3.1*	610	9.8*

* as N,P or S

FIG.2: ARTIFICIAL STREAM APPARATUS: PLAN



2.6.2 Artificial stream channels

Periphytic algae were cultured in the laboratory under conditions approximating those in the field, with control of water chemistry, temperature, flow rate and light regime. A plan of the apparatus used is shown in Fig. 2.

Artificial stream channels were constructed using clear Perspex sheet. Water is pumped in at one end by means of a submersible aquarium pump (Aquaclear 200) in a 25 litre polythene bucket, connected to the channel by 10mm i.d. silicone tubing. Filtered air is pumped in by a Whisper 600 air pump at the same end of the channel via a perforated plastic pipe, so that the channel water is in equilibrium with the atmosphere in respect of dissolved gases. The water leaves the channel at the other end via a 20mm tube and returns to the bucket via a channel constructed out of 40mm plastic electrical trunking. Six buckets are accommodated in a circular black PVC water tank, supported on a platform of galvanized Dexion and immersed to approximately two-thirds their depth in water. This is thermostatically contolled by means of a thermistor and associated circuitry (see Appendix 3) connected to a Grant CZ2 dip cooler unit. Stirring is also provided in order to prevent icing up of the cooling coils. During the course of this work a temperature of 10°C was employed, and manual checks reveal that the temperature varies by less than ±0.25°C.

The submersible pumps are not fully insulated and it has been found that some generate a potential of up to 60V between the stream water and ground, which causes problems when using a pH electrode in the channels. The buckets are therefore grounded by means of stainless steel rods dipping into the water. The Dexion frame of the apparatus, and the submerged Dexion platform are also connected to ground.

Light is provided by two 40W cool white fluorescent tubes 40cm above the channels. The light regime used was 12h light : 12h dark, controlled by a time switch. Natural light is excluded from the whole apparatus by enclosing it in a black polythene tent, and light from the tubes is likewise excluded from the tank and buckets. To further minimise the possibility of algal growth outwith the channels, the outside of the buckets and lids and the return channels are painted black.

The buckets used are 25 litre food grade polythene (HDPE) brewing bins (Cumbria Brew Bins). They were pre-treated to remove excess plasticiser which might be inhibitory to algal growth (Hardwick and Cole, 1986) by twice steaming them full of distilled water at 100°C for 1 hour in an autoclave (higher temperatures result in shrinkage and distortion of the HDPE).

Channel pH was controlled manually by addition of 0.2N H_2SO_4. Growth experiments were carried out in pH-adjusted Corrie medium. This requires checking and adjustment about once every two days to maintain reasonable control of pH as the pH tends to rise with time especially at higher nominal pH values. In unadjusted Corrie medium, the pH may rise to over 7.0 on continued stirring. This may be due to the slow dissolution of Al complexes or Al hydroxides (Tipping *et al*, 1989). The B9 medium on the other hand gives an unadjusted pH of 4.6-4.7, which remains constant.

Algae were grown attached to slides which were held down on the bottom of the channels by Perspex grips. The slides were seeded from field samples. Some species readily generate zoospores while others will regenerate from hormogonia; these processes may be encouraged by a shift from a phosphate-depleted to a phosphate-enriched medium (Rosemarin, 1983). This was accomplished by placing slides in the bottom of a Pyrex tray, covered with 1 litre of B9 medium, which is high in PO_4, as compared with Corrie (Table 3). Algae may be added directly to the medium, or in a 1mm mesh container suspended within it. Aeration and stirring were supplied by means of small airlift pumps constructed out of a Pasteur pipette and 10mm glass tubing, connected to an aquarium air pump (Whisper 600). After a period of settlement of 1-6 days (depending on the density required) the slides were removed, rinsed lightly, and transferred to other media.

2.6.3 Measurement of growth rates

Growth was measured by direct cell counting of individual plants (Rosemarin, 1983) at intervals of 2-4 days. This method can be used only at low algal densities and at an early phase of growth; 3 or 4 successive measurements may be made of each filament. Up to 4 main species were grown simultaneously on the same slide, and 3 slides were employed per channel.

2.6.4 Assessment of relative abundance

The relative abundance of species in channels was scored in the same manner as was used with field samples, allowing assessments to be made of the development and changes of dominance on prolonged culture.

2.6.5 Static and semi-static culture

Batch culture of isolates was carried out for the purpose of performing identifications (Cox and Bold, 1966) providing inocula or to test the effect of changes in the growth medium, in Ehrlenmeyer flasks or in 2 litre square plastic tubs (2Kg margarine tubs). If stirring or aeration was required an airlift pump was employed as described earlier. Incubation was carried out either in a waterbath illuminated from above or in an illuminated environmental chamber (Baird and Tatlock cooled incubator with side or bottom lighting).

2.7 STATISTICAL METHODS

Correlation and linear regression, manipulation of data and analysis of variance was carried out using MINITAB on a Prime 9955 mini computer.

Statistical ecology programs to carry out the analyses described by Ludwig and Reynolds (1988) are available on the disk supplied with the book and these were used to carry out Cluster Analysis, Normal Association Analysis and calculation of diversity indices.

Canonical Correspondence Analysis and its detrended form were carried out using the programme CANOCO (Ter Braak, 1988). SIGMAPLOT (Jandel Scientific) was used to prepare plots.

2.7.1 Recalculation of species and site values from CANOCO

The species and site values with respect to an environmental variable can be obtained by dropping a perpendicular to the arrow or its backwards extension from each species or site point in the ordination diagram (Ter Braak, 1987). This is more accurately carried out from the species coordinates and the coordinates of the head of the variable's arrow using the formula:-

$$\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} \cos\theta & \sin\theta \\ -\sin\theta & \cos\theta \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix}$$

where $\tan\theta = x_v / y_v$ (x_v = x coordinate of the arrow head;
y_v = y coordinate of the arrow head)

3. RESULTS

3.1 FIELD SAMPLING: SPECIES DATA

Forty-nine taxa of filamentous algae were distinguished in field samples. Descriptions and some tentative identifications are presented on Appendix 1. Diatoms, desmids and unicellular green algae were also found in samples but the data and analyses presented here are based solely on filamentous algae.

The fifteen sampling sites were visited on up to thirty-five occasions. The species abundance data thus constitute a three-dimensional matrix (49 species, 15 sites, 35 sampling dates). The matrix has been divided into two almost equal parts for comparative purposes. Some of the sites were not sampled prior to sampling number 17 or 18, and thus the data obtained subsequently are more complete. All sampling occasions up to no. 17 have been combined into Matrix A, and numbers 18 to 35 inclusive comprise Matrix B. The undivided matrix has also been subjected to analyses, as Matrix C. The environmental and biomass data have been divided along similar lines. The 3-dimensional matrices must be reduced to a 2-D form to enable most statistical procedures to be carried out. This reduction was accomplished by calculating the mean relative abundance of each species in each site, taking into account the number of times each site was sampled. In the terminology of Ludwig and Reynolds (1988) a site constitutes a 'Sampling Unit' (S.U.).

The species data may also be used as presence-absence data in some statistical procedures. Conversion of the abundance data to presence-absence (p/a) was carried out in MINITAB by converting all non-zero values to 1. This procedure was carried out in each of the 2-D matrices. Conversion of the 3-D matrices was also carried out. If the mean (sum of all presences divided by the number of samples taken) of the p/a data in the 3-D matrices is calculated, a measure of relative abundance of species is obtained which is not dependent upon estimates of relative abundance in each sample. The data obtained in this way, termed 'pooled p/a' have been used in the same analyses as the abundance data.

Species data matrices A, B and C in both mean abundance and pooled p/a form are shown in Tables 4 to 9. The means have been multiplied by 100 to round up to whole numbers, as required for analysis by the Ludwig and Reynolds (1988) programs.

TABLE 4

MEAN VALUES (ROUNDED) OF RELATIVE SPECIES ABUNDANCE FOR EACH SITE, MATRIX A

	SPECIES	SITE													
		14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
1	Oscillatoria 1um.	0	0	0	0	0	0	0	0	0	0	0	12	44	0
2	Oscillatoria 2um.	0	0	0	0	20	0	0	0	0	0	0	29	0	100
3	Oscillatoria 5um.	0	40	0	7	13	0	0	0	0	0	0	53	94	400
4	Oscillatoria 8um.	0	100	0	0	13	0	0	0	0	0	0	12	206	0
5	Oscillatoria 11um.	0	0	0	0	7	0	0	0	0	0	0	112	156	0
6	Oscillatoria 14um.	0	0	0	0	0	0	0	0	0	0	0	29	13	0
7	Phormidium 3um.	0	20	0	27	0	0	0	0	0	0	0	12	69	600
8	Phormidium 5um.	0	70	0	7	193	0	0	0	0	0	0	388	313	0
9	Phormidium 8um.	0	0	0	0	0	0	0	0	0	0	0	0	44	0
10	Tolypothrix	0	70	0	0	420	0	0	8	0	0	0	6	6	0
11	Toly.epiphyte	0	0	0	0	107	0	0	0	0	0	0	0	0	0
12	Stigonema	0	0	33	0	13	0	0	0	0	0	0	0	0	0
13	Scytonema	0	0	0	0	140	300	0	0	0	0	0	0	313	0
14	Batrachospermum	0	40	0	7	27	50	0	0	0	0	0	129	200	600
15	Audouinella	0	40	7	0	0	0	0	0	0	0	0	0	0	0
16	Draparnaldia	0	120	0	27	120	0	0	0	0	0	0	77	263	0
17	Stigeoclonium 5um.	139	0	87	267	180	300	406	246	156	142	282	18	94	0
18	Stigeoclonium 8um.	0	10	13	13	0	0	0	0	0	0	0	106	13	200
19	Oedogonium 6um.	0	120	100	213	140	0	0	15	0	0	12	6	144	0
20	Oedogonium 14um.	0	210	0	0	20	0	0	0	0	0	0	35	69	100
21	Oedogonium 30um.	0	0	0	0	0	0	0	8	0	0	0	6	75	600
22	Spirogyra 20um.	0	0	0	0	0	0	0	0	0	0	0	0	44	0
23	Spirogyra 32um.	0	10	0	0	0	0	0	0	0	0	0	18	6	100
24	Zygnema 20um.	0	50	7	20	360	0	0	8	0	0	0	0	6	0
25	Zygnema 28um.	0	30	0	0	0	0	0	0	0	0	0	0	0	0
26	Mougeotia 5um.	31	50	80	147	147	0	59	162	67	133	100	71	181	0
27	Mougeotia 11um.	185	50	240	333	247	0	153	115	100	142	94	24	125	0
28	Mougeotia 16um.	8	20	20	27	87	0	12	77	44	192	147	18	100	0
29	Mougeotia 20um.	0	30	87	0	7	0	0	0	0	17	24	0	81	0
30	Mougeotia 25um.	0	20	13	47	0	0	0	0	0	0	0	47	138	0
31	Microthamnion	8	10	0	0	0	0	41	0	11	42	18	18	38	0
32	Microspora 8um.	131	40	220	293	147	0	147	92	133	183	300	35	63	0
33	Microspora 11um.	31	0	27	7	0	0	0	15	0	17	6	6	0	0
34	Microspora 14um.	0	0	33	0	0	0	59	139	56	242	53	0	0	0
35	Microspora 22um.	0	0	0	7	0	0	0	0	0	0	0	0	0	0
36	Geminella 8um.	177	20	300	253	93	0	47	123	144	167	71	159	181	0
37	Geminella 11um.	39	0	20	107	40	0	0	0	0	25	0	0	144	0
38	Hormidium 5um.	508	300	527	227	340	50	382	385	267	92	482	241	125	200
39	Hormidium 8um.	239	220	113	53	27	0	129	123	56	75	200	29	44	0
40	Ulothrix 8um.	54	60	13	7	53	0	18	23	11	17	12	35	31	0
41	Ulothrix 11um.	0	0	0	33	7	0	71	31	11	0	12	12	13	100
42	Ulo./scaly 11um.	8	80	20	27	0	0	0	8	0	0	0	41	0	0
43	Ulothrix 14um.	0	0	0	0	0	0	0	0	0	0	0	0	6	0
44	Ulothrix 17um.	0	0	0	0	7	0	0	0	0	0	0	0	0	100

TABLE 5

MEAN VALUES (ROUNDED) OF RELATIVE SPECIES ABUNDANCE FOR EACH SITE, MATRIX B

	SPECIES	SITE														
		14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
1	Oscillatoria 1um.	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0
2	Oscillatoria 2um.	0	0	19	0	0	17	18	0	0	0	0	0	13	22	41
3	Oscillatoria 5um.	0	0	19	0	0	22	0	0	0	0	0	0	133	267	129
4	Oscillatoria 8um.	0	0	94	0	0	22	27	0	0	0	0	0	60	106	59
5	Oscillatoria 11um.	0	0	0	0	0	0	0	0	0	0	0	0	27	178	94
6	Oscillatoria 14um.	0	0	0	0	0	0	0	0	0	0	0	0	0	61	35
7	Phormidium 3um.	0	0	0	0	0	11	0	0	0	0	0	0	13	0	112
8	Phormidium 5um.	0	0	456	0	11	306	55	0	0	0	0	0	240	433	247
9	Phormidium 8um.	0	0	0	0	0	67	0	0	0	0	0	0	227	72	0
10	Phormidium 13um.	0	0	0	0	0	0	0	0	0	0	0	0	20	6	0
11	Tolypothrix	0	0	250	0	56	656	309	0	6	0	7	0	0	44	82
12	Toly.epiphyte	0	0	19	0	0	289	82	0	0	0	0	0	0	0	0
13	Stigonema	56	22	44	229	0	106	273	0	0	0	0	0	0	0	0
14	Scytonema	0	0	6	47	0	0	0	0	0	0	0	0	0	0	0
15	Batrachospermum	0	0	0	288	139	222	391	0	0	0	0	6	60	0	0
16	Lemanea	6	0	494	0	0	317	0	0	0	0	0	0	0	422	394
17	Audouinella	31	0	44	12	17	6	0	6	6	11	0	0	213	550	547
18	Audouinella(green)	6	0	31	6	0	33	27	0	0	0	0	0	7	28	35
19	Draparnaldia	0	0	38	0	44	283	0	0	0	0	0	0	120	350	6
20	Stigeoclonium 5um.	106	111	0	200	328	78	55	600	194	200	36	111	20	0	12
21	Stigeoclonium 8um.	0	0	31	88	72	89	136	0	0	0	0	0	20	83	371
22	Oedogonium 6um.	13	0	25	388	261	261	127	0	0	6	0	6	0	56	53
23	Oedogonium 14um.	6	0	369	12	17	100	9	0	0	0	0	0	7	72	47
24	Oedogonium 30um.	13	0	38	0	0	17	0	0	0	0	0	0	0	333	394
25	Spirogyra 20um.	0	0	0	0	17	11	0	0	0	0	0	0	0	78	118
26	Spirogyra 32um.	0	0	0	0	0	0	0	0	0	0	0	0	0	122	18
27	Zygnema 20um.	0	0	6	159	250	544	246	0	0	0	0	0	0	11	0
28	Zygnema 28um.	0	0	81	12	0	17	0	0	0	0	0	0	0	0	0
29	Mougeotia 5um.	56	22	19	59	44	100	9	11	29	33	14	17	0	94	12
30	Mougeotia 11um.	363	300	75	388	294	350	209	111	47	28	43	72	27	128	47
31	Mougeotia 16um.	31	0	6	177	22	78	27	0	0	0	21	28	0	28	0
32	Mougeotia 20um.	13	22	38	218	0	28	0	0	0	0	0	6	0	94	47
33	Mougeotia 25um.	0	0	13	12	17	94	0	0	0	0	0	0	7	100	59
34	Microthamnion	0	0	0	0	6	0	0	22	12	11	7	6	0	0	0
35	Microspora 8um.	225	333	6	359	294	133	209	83	77	33	93	511	0	78	41
36	Microspora 11um.	6	67	0	18	0	0	27	6	0	0	0	6	0	28	0
37	Microspora 14um.	6	0	0	47	0	0	27	0	0	6	314	144	0	28	6
38	Microspora 22um.	0	0	0	0	0	0	0	0	0	0	0	0	167	256	153
39	Geminella 8um.	394	289	0	453	228	133	36	39	65	61	36	33	120	183	53
40	Geminella 11um.	38	33	0	82	111	28	46	0	6	11	7	0	20	72	29
41	Hormidium 5um.	456	444	269	418	450	456	409	450	371	522	207	528	320	106	124
42	Hormidium 8um.	125	233	194	147	117	78	36	183	53	161	100	250	40	22	94
43	Ulothrix 8um.	0	0	44	41	0	33	0	28	47	0	29	56	0	39	6
44	Ulothrix 11um.	0	11	75	65	6	11	0	33	0	6	0	0	93	6	59
45	Ulo./scaly 11um.	63	67	31	47	17	22	18	0	35	33	7	0	67	11	12
46	Ulothrix 14um.	0	0	0	0	6	6	0	0	0	0	0	0	0	6	88
47	Ulothrix 17um.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	112
48	Ulothrix 28um.	0	0	6	0	0	0	0	0	0	0	0	0	0	0	135
49	Ulothrix 36um.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65

TABLE 6

MEAN VALUES (ROUNDED) OF RELATIVE SPECIES ABUNDANCE FOR EACH SITE, MATRIX C

	SPECIES	SITE														
		14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
1	Oscillatoria 1um.	0	0	0	0	0	0	0	3	0	0	0	0	6	21	0
2	Oscillatoria 2um.	0	0	12	0	0	18	15	0	0	0	0	0	22	12	44
3	Oscillatoria 5um.	0	0	27	0	3	18	0	0	0	0	0	0	91	185	144
4	Oscillatoria 8um.	0	0	96	0	0	18	23	0	0	0	0	0	34	153	56
5	Oscillatoria 11um.	0	0	0	0	0	3	0	0	0	0	0	0	72	168	89
6	Oscillatoria 14um.	0	0	0	0	0	0	0	0	0	0	0	0	16	38	33
7	Phormidium 3um.	0	0	8	0	12	6	0	0	0	0	0	0	13	32	139
8	Phormidium 5um.	0	0	308	0	9	255	46	0	0	0	0	0	319	377	233
9	Phormidium 8um.	0	0	0	0	0	36	0	0	0	0	0	0	106	59	0
10	Phormidium 13um.	0	0	0	0	0	0	0	0	0	0	0	0	9	3	0
11	Tolypothrix	0	0	181	0	30	549	262	0	7	0	4	0	3	27	78
12	Toly.epiphyte	0	0	12	0	0	206	69	0	0	0	0	0	0	0	0
13	Stigonema	56	9	27	138	0	64	231	0	0	0	0	0	0	0	0
14	Scytonema	0	0	4	25	0	0	0	0	0	0	0	0	0	0	0
15	Batrachospermum	0	0	0	153	76	185	377	0	0	0	0	3	28	147	0
16	Lemanea	6	0	304	0	0	173	0	0	0	0	0	0	0	224	372
17	Audouinella	31	0	42	6	12	15	8	3	3	7	0	0	169	385	550
18	Audouinella(green)	6	0	35	6	0	18	23	0	0	0	0	0	3	15	33
19	Draparnaldia	0	0	69	0	36	209	0	0	0	0	0	0	97	309	6
20	Stigeoclonium 5um.	106	127	0	147	300	124	92	506	217	185	85	194	19	44	11
21	Stigeoclonium 8um.	0	0	23	53	46	49	115	0	0	0	0	0	66	50	361
22	Oedogonium 6um.	13	0	62	253	239	206	108	0	7	4	0	9	3	97	50
23	Oedogonium 14um.	6	0	308	6	9	64	8	0	0	0	0	0	22	71	50
24	Oedogonium 30um.	13	0	23	0	0	9	0	0	3	0	0	0	3	212	406
25	Spirogyra 20um.	0	0	0	0	9	6	0	0	0	0	0	0	0	62	111
26	Spirogyra 32um.	0	0	4	0	0	0	0	0	0	0	0	0	9	68	22
27	Zygnema 20um.	0	0	23	88	146	461	208	0	3	0	0	0	0	9	0
28	Zygnema 28um.	0	0	62	6	0	9	0	0	0	0	0	0	0	0	0
29	Mougeotia 5um.	56	27	31	69	91	121	8	34	87	44	69	57	38	135	11
30	Mougeotia 11um.	363	232	65	319	312	303	177	131	77	52	89	83	25	127	44
31	Mougeotia 16um.	31	5	12	103	24	82	23	6	33	15	100	86	9	62	0
32	Mougeotia 20um.	13	9	35	156	0	18	0	0	0	0	8	14	0	88	44
33	Mougeotia 25um.	0	0	15	13	30	52	0	0	0	0	0	0	28	118	56
34	Microthamnion	0	5	4	0	3	0	0	31	7	11	23	11	9	18	0
35	Microspora 8um.	225	214	19	294	294	139	177	114	83	67	135	409	19	71	39
36	Microspora 11um.	6	46	0	22	3	0	23	3	7	0	8	6	3	15	0
37	Microspora 14um.	6	0	0	41	0	0	23	29	60	22	281	100	0	15	6
38	Microspora 22um.	0	0	0	0	3	0	0	0	0	0	0	0	78	135	144
39	Geminella 8um.	394	223	8	381	239	115	31	43	90	89	96	51	141	182	50
40	Geminella 11um.	38	36	0	53	109	33	39	0	3	7	15	0	9	106	28
41	Hormidium 5um.	456	482	281	469	349	403	354	417	377	437	154	506	278	115	128
42	Hormidium 8um.	125	236	204	131	88	55	31	157	83	126	89	226	34	32	89
43	Ulothrix 8um.	0	32	50	28	3	42	0	23	37	4	23	34	19	35	6
44	Ulothrix 11um.	0	5	46	34	18	9	0	51	13	7	0	6	50	9	61
45	Ulo./scaly 11um.	63	32	50	34	21	12	15	0	23	22	4	0	53	6	11
46	Ulothrix 14um.	0	0	0	0	3	3	0	0	0	0	0	0	0	6	83
47	Ulothrix 17um.	0	0	0	0	0	3	0	0	0	0	0	0	0	0	111
48	Ulothrix 28um.	0	0	4	0	0	0	0	0	0	0	0	0	0	0	128
49	Ulothrix 36um.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	61

TABLE 7

VALUES OF POOLED SPECIES PRESENCE-ABSENCE FOR EACH SITE, MATRIX A

	SPECIES	SITE													
		14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
1	Oscillatoria 1um.	0	0	0	0	0	0	0	0	0	0	0	2	2	0
2	Oscillatoria 2um.	0	0	0	0	3	0	0	0	0	0	0	2	0	1
3	Oscillatoria 5um.	0	3	0	1	2	0	0	0	0	0	0	6	5	1
4	Oscillatoria 8um.	0	5	0	0	2	0	0	0	0	0	0	2	10	0
5	Oscillatoria 11um.	0	0	0	0	1	0	0	0	0	0	0	8	11	0
6	Oscillatoria 14um.	0	0	0	0	0	0	0	0	0	0	0	2	1	0
7	Phormidium 3um.	0	1	0	1	0	0	0	0	0	0	0	1	4	1
8	Phormidium 5um.	0	4	0	1	6	0	0	0	0	0	0	12	11	0
9	Phormidium 8um.	0	0	0	0	0	0	0	0	0	0	0	0	2	0
10	Tolypothrix	0	2	0	0	13	0	0	1	0	0	0	1	1	0
11	Toly.epiphyte	0	0	0	0	5	0	0	0	0	0	0	0	0	0
12	Stigonema	0	0	2	0	2	0	0	0	0	0	0	0	0	0
13	Scytonema	0	0	0	0	4	1	0	0	0	0	0	0	9	0
14	Batrachospermum	0	2	0	1	3	1	0	0	0	0	0	8	10	1
15	Audouinella	0	1	1	0	0	0	0	0	0	0	0	0	0	0
16	Draparnaldia	0	2	0	1	3	0	0	0	0	0	0	2	10	0
17	Stigeoclonium 5um.	6	0	7	13	9	1	14	7	4	7	10	2	4	0
18	Stigeoclonium 8um.	0	1	2	1	0	0	0	0	0	0	0	3	2	1
19	Oedogonium 6um.	0	5	8	11	10	0	0	1	0	0	1	1	9	0
20	Oedogonium 14um.	0	4	0	0	2	0	0	0	0	0	0	3	4	1
21	Oedogonium 30um.	0	0	0	0	0	0	0	1	0	0	0	1	4	1
22	Spirogyra 20um.	0	0	0	0	0	0	0	0	0	0	0	0	4	0
23	Spirogyra 32um.	0	1	0	0	0	0	0	0	0	0	0	3	1	1
24	Zygnema 20um.	0	2	1	1	12	0	0	1	0	0	0	0	1	0
25	Zygnema 28um.	0	3	0	0	0	0	0	0	0	0	0	0	0	0
26	Mougeotia 5um.	3	3	7	6	9	0	5	7	4	7	7	5	10	0
27	Mougeotia 11um.	5	5	10	12	14	0	8	8	4	6	6	3	8	0
28	Mougeotia 16um.	1	2	3	4	4	0	1	3	3	8	7	1	7	0
29	Mougeotia 20um.	0	2	5	0	1	0	0	0	0	2	2	0	6	0
30	Mougeotia 25um.	0	2	1	2	0	0	0	0	0	0	0	4	8	0
31	Microthamnion	1	1	0	0	0	0	4	0	1	3	3	2	3	0
32	Microspora 8um.	7	1	12	11	10	0	8	5	3	7	14	5	4	0
33	Microspora 11um.	1	0	2	1	0	0	0	1	0	2	1	1	0	0
34	Microspora 14um.	0	0	2	0	0	0	3	3	2	8	5	0	0	0
35	Microspora 22um.	0	0	0	1	0	0	0	0	0	0	0	0	0	0
36	Geminella 8um.	8	1	12	10	10	0	5	7	3	7	5	8	9	0
37	Geminella 11um.	2	0	3	6	3	0	0	0	0	1	0	0	8	0
38	Hormidium 5um.	13	7	15	13	12	1	16	11	7	5	16	12	11	1
39	Hormidium 8um.	8	4	5	3	1	0	6	4	2	4	6	3	2	0
40	Ulothrix 8um.	2	3	2	1	2	0	3	2	1	2	2	4	4	0
41	Ulothrix 11um.	0	0	0	2	1	0	2	1	1	0	1	1	2	1
42	Ulo./scaly 11um.	1	4	2	1	0	0	0	1	0	0	0	2	0	0
43	Ulothrix 14um.	0	0	0	0	0	0	0	0	0	0	0	0	1	0
44	Ulothrix 17um.	0	0	0	0	1	0	0	0	0	0	0	0	0	1

TABLE 8

VALUES OF POOLED SPECIES PRESENCE-ABSENCE FOR EACH SITE, MATRIX B

	SPECIES	SITE														
		14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
1	Oscillatoria 1um.	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
2	Oscillatoria 2um.	0	0	1	0	0	3	1	0	0	0	0	0	2	1	3
3	Oscillatoria 5um.	0	0	3	0	0	4	0	0	0	0	0	0	4	16	11
4	Oscillatoria 8um.	0	0	9	0	0	2	1	0	0	0	0	0	4	11	5
5	Oscillatoria 11um.	0	0	0	0	0	0	0	0	0	0	0	0	4	12	7
6	Oscillatoria 14um.	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3
7	Phormidium 3um.	0	0	0	0	0	1	0	0	0	0	0	0	1	0	4
8	Phormidium 5um.	0	0	14	0	2	10	1	0	0	0	0	0	7	13	10
9	Phormidium 8um.	0	0	0	0	0	2	0	0	0	0	0	0	7	3	0
10	Phormidium 13um.	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
11	Tolypothrix	0	0	9	0	5	18	8	0	1	0	1	0	0	6	5
12	Toly.epiphyte	0	0	1	0	0	12	3	0	0	0	0	0	0	0	0
13	Stigonema	2	1	3	7	0	4	9	0	0	0	0	0	0	0	0
14	Scytonema	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0
15	Batrachospermum	0	0	0	8	5	9	8	0	0	0	0	1	2	0	0
16	Lemanea	1	0	14	0	0	12	0	0	0	0	0	0	0	14	13
17	Audouinella	2	0	3	2	3	1	0	1	1	2	0	0	9	17	16
18	Audouinella(green)	1	0	2	1	0	4	1	0	0	0	0	0	1	4	5
19	Draparnaldia	0	0	1	0	3	10	0	0	0	0	0	0	3	11	1
20	Stigeoclonium 5um.	5	4	0	11	13	7	2	16	7	10	3	5	1	0	1
21	Stigeoclonium 8um.	0	0	1	4	3	5	5	0	0	0	0	0	2	11	13
22	Oedogonium 6um.	2	0	4	16	17	15	7	0	0	1	0	1	0	6	4
23	Oedogonium 14um.	1	0	12	2	2	8	1	0	0	0	0	0	1	5	4
24	Oedogonium 30um.	1	0	3	0	0	3	0	0	0	0	0	0	0	14	15
25	Spirogyra 20um.	0	0	0	0	1	2	0	0	0	0	0	0	0	4	5
26	Spirogyra 32um.	0	0	0	0	0	0	0	0	0	0	0	0	0	7	2
27	Zygnema 20um.	0	0	1	10	11	16	8	0	0	0	0	0	0	2	0
28	Zygnema 28um.	0	0	4	1	0	2	0	0	0	0	0	0	0	0	0
29	Mougeotia 5um.	6	2	3	6	5	9	1	2	4	4	2	3	0	10	1
30	Mougeotia 11um.	13	7	6	15	15	15	8	11	5	3	6	4	2	14	8
31	Mougeotia 16um.	5	0	1	13	4	8	2	0	0	0	2	5	0	5	0
32	Mougeotia 20um.	2	1	5	13	0	4	0	0	0	0	0	1	0	8	7
33	Mougeotia 25um.	0	0	1	2	3	9	0	0	0	0	0	0	1	7	3
34	Microthamnion	0	0	0	0	1	0	0	3	2	1	1	1	0	0	0
35	Microspora 8um.	13	6	1	15	14	15	6	7	5	4	5	16	0	5	2
36	Microspora 11um.	1	1	0	2	0	0	1	1	0	0	0	1	0	2	0
37	Microspora 14um.	1	0	0	5	0	0	1	0	0	1	7	9	0	3	1
38	Microspora 22um.	0	0	0	0	0	0	0	0	0	0	0	0	4	9	5
39	Geminella 8um.	15	6	0	15	16	10	2	6	5	5	5	5	3	13	8
40	Geminella 11um.	3	2	0	8	10	5	2	0	1	1	1	0	3	5	5
41	Hormidium 5um.	16	8	9	17	16	17	10	17	12	18	9	16	12	10	6
42	Hormidium 8um.	7	4	6	5	6	3	2	6	4	7	3	9	1	1	3
43	Ulothrix 8um.	0	0	2	2	0	1	0	3	2	0	1	3	0	2	1
44	Ulothrix 11um.	0	1	3	3	1	2	0	2	0	1	0	0	3	1	3
45	Ulo./scaly 11um.	2	1	2	3	2	1	2	0	4	2	1	0	3	1	2
46	Ulothrix 14um.	0	0	0	0	1	1	0	0	0	0	0	0	0	1	7
47	Ulothrix 17um.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
48	Ulothrix 28um.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	6
49	Ulothrix 36um.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3

TABLE 9

VALUES OF POOLED SPECIES PRESENCE-ABSENCE FOR EACH SITE, MATRIX C

	SPECIES	SITE														
		14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
1	Oscillatoria 1um.	0	0	0	0	0	0	0	1	0	0	0	0	2	2	0
2	Oscillatoria 2um.	0	0	1	0	0	6	1	0	0	0	0	0	4	1	4
3	Oscillatoria 5um.	0	0	6	0	1	6	0	0	0	0	0	0	10	21	12
4	Oscillatoria 8um.	0	0	14	0	0	4	1	0	0	0	0	0	6	21	5
5	Oscillatoria 11um.	0	0	0	0	0	1	0	0	0	0	0	0	12	23	7
6	Oscillatoria 14um.	0	0	0	0	0	0	0	0	0	0	0	0	2	5	3
7	Phormidium 3um.	0	0	1	0	1	1	0	0	0	0	0	0	2	4	5
8	Phormidium 5um.	0	0	18	0	3	16	1	0	0	0	0	0	19	24	10
9	Phormidium 8um.	0	0	0	0	0	2	0	0	0	0	0	0	7	5	0
10	Phormidium 13um.	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
11	Tolypothrix	0	0	11	0	5	31	8	0	2	0	1	0	1	7	5
12	Toly.epiphyte	0	0	1	0	0	17	3	0	0	0	0	0	0	0	0
13	Stigonema	2	1	3	9	0	6	9	0	0	0	0	0	0	0	0
14	Scytonema	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0
15	Batrachospermum	0	0	0	8	5	13	9	0	0	0	0	1	2	9	0
16	Lemanea	1	0	14	0	0	12	0	0	0	0	0	0	0	14	13
17	Audouinella	2	0	5	2	4	4	1	1	1	2	0	0	17	27	17
18	Audouinella(green)	1	0	3	2	0	4	1	0	0	0	0	0	1	4	5
19	Draparnaldia	0	0	3	0	4	13	0	0	0	0	0	0	5	21	1
20	Stigeoclonium 5um.	5	10	0	18	26	16	3	30	14	14	10	15	3	4	1
21	Stigeoclonium 8um.	0	0	2	6	4	5	5	0	0	0	0	0	5	13	14
22	Oedogonium 6um.	2	0	9	24	28	25	7	0	1	1	0	2	1	15	4
23	Oedogonium 14um.	1	0	16	2	2	10	1	0	0	0	0	0	4	9	5
24	Oedogonium 30um.	1	0	3	0	0	3	0	0	1	0	0	0	1	18	16
25	Spirogyra 20um.	0	0	0	0	1	2	0	0	0	0	0	0	0	8	5
26	Spirogyra 32um.	0	0	1	0	0	0	0	0	0	0	0	0	3	8	3
27	Zygnema 20um.	0	0	3	11	12	28	8	0	1	0	0	0	0	3	0
28	Zygnema 28um.	0	0	7	1	0	2	0	0	0	0	0	0	0	0	0
29	Mougeotia 5um.	6	5	6	13	11	18	1	7	11	8	9	10	5	20	1
30	Mougeotia 11um.	13	12	11	25	27	29	8	19	13	7	12	10	5	22	8
31	Mougeotia 16um.	5	1	3	16	8	12	2	1	3	3	10	12	1	12	0
32	Mougeotia 20um.	2	1	7	18	0	5	0	0	0	0	2	3	0	14	7
33	Mougeotia 25um.	0	0	3	3	5	9	0	0	0	0	0	0	5	15	3
34	Microthamnion	0	1	1	0	1	0	0	7	2	2	4	4	2	3	0
35	Microspora 8um.	13	13	2	27	25	25	6	15	10	7	12	30	5	9	2
36	Microspora 11um.	1	2	0	4	1	0	1	1	1	0	2	2	1	2	0
37	Microspora 14um.	1	0	0	7	0	0	1	3	3	3	15	14	0	3	1
38	Microspora 22um.	0	0	0	0	1	0	0	0	0	0	0	0	4	9	5
39	Geminella 8um.	15	14	1	27	26	20	2	11	12	8	12	10	11	22	8
40	Geminella 11um.	3	4	0	11	16	8	2	0	1	1	2	0	3	13	5
41	Hormidium 5um.	16	21	16	32	29	29	11	33	23	25	14	32	24	21	7
42	Hormidium 8um.	7	12	10	10	9	4	2	12	8	9	7	15	4	3	3
43	Ulothrix 8um.	0	2	5	4	1	3	0	6	4	1	3	5	4	6	1
44	Ulothrix 11um.	0	1	3	3	3	3	0	4	1	2	0	1	4	3	4
45	Ulo./scaly 11um.	2	2	6	5	3	1	2	0	5	2	1	0	5	1	2
46	Ulothrix 14um.	0	0	0	0	1	1	0	0	0	0	0	0	0	2	7
47	Ulothrix 17um.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	8
48	Ulothrix 28um.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	6
49	Ulothrix 36um.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3

3.2 ENVIRONMENTAL DATA

Characteristics of the sites are shown in Table 1. Site characteristics 2 to 9 and 15 or16 have been used in subsequent analysis by CANOCO.

Mean values for environmental variables in the first and second halves of the sampling period (matrices A and B), and over the whole period (Matrix C), are shown in Tables 10 to 12.

The environmental data obtained during the study has been supplemented by data obtained by SOAFD, Freshwater Fisheries Laboratory, Pitlochry. The latter data were obtained from composite water samples and thus integrate the values of the chemical determinands over a 1 week period. For each algal sampling occasion the mean value of water chemistry variables since the previous sample has been calculated.

Comparison of pH and optical absorbance data obtained from composite samples with data from spot samples taken at the same time as algal samples reveal similar values but with the composite samples yielding a smaller range. These represent a better approximation to the true (time-averaged) value. Both pH from spot samples (instantaneous pH = pH INST) and from composite samples (pH COMP) have been used in the investigation of species-environment relationships.

The individual sample data have been used in linear correlation analyses with species and biomass data. All other analyses use the site-average values. Some correlation analyses have also been carried out with site-average values.

There is very close agreement between instantaneous and composite pH (and hence H^+). Mean pH is calculated from mean H^+ (Jordan, 1989), rather than as the arithmetic mean.

The 'Flow' measurements are instantaneous readings of a depth gauge and do not allow a direct measure of current velocity since this depends also on other hydrological factors. Furthermore they are not directly comparable between different sites. An attempt has been made to compensate for a possible cause of differences in mean depth gauge reading, by subtracting the lowest recorded reading at each site. This removes inaccuracy due to build-up of sediments on the stream bed, since this measurement was made under extremely low-flow conditions. These corrected values, termed 'Depth' in the Tables and Diagrams, in fact show the mean variation in stream depth, a measure of the susceptibility to large changes in discharge. 'Flow' includes a constant element for each site, which may introduce a spurious correlation with other environmental factors.

Both 'Flow' and 'Depth' have been included in analyses in order to investigate this possibility.

Instantaneous temperature is liable to bias, as discussed in Section 4.4.2. The measurements of maximum and minimum temperatures are too few to be of value in statistical analyses.

Absorbance measurements have been used to infer values for Total Organic Carbon TOC) in natural waters (Edwards and Cresser, 1987; Rees *et al*, 1989). These authors utilized absorbance at 350 nm, while others have used A_{350} as a close correlate with TOC (Karlsson *et al*, 1987; Gjessing *et al*, 1989). The Freshwater Fisheries Laboratory have routinely measured A_{400} and A_{250} and this practice was continued in this work. Measurements of TOC made in the L. Ard streams are highly correlated with A_{250} (R. Harriman, pers. comm.) A_{400} is a measure of turbidity of the water and is positively correlated with A_{250} in field samples.

Mean (24 hour) Photosynthetically Available Radiation (PAR) was measured over several time intervals at 8 sites, allowing calculation of a mean value for integrated PAR as a percentage of that available at the control 'open' site on Burn 6. These mean values have been assumed to remain constant over the whole sampling period. For analyses by CANOCO the percentages have been used, with estimated values for those sites not actually measured. For correlation analysis utilizing individual sample data, values of incident PAR immediately prior to the sampling, were calculated from the measured incident PAR at Burn 6 and the percentage values. The means of these values are shown in Tables 10-12 as 'absolute PAR' (= PAR ABS).

Measurements of PAR could not be carried out at all sites because of the limited number of instruments available (five) and the possibility of interference occurring at some sites. Therefore subjective estimates have been made in order to enable CANOCO to carry out analyses incorporating data on PAR. These however have not been used in correlation analyses (Table 13).

Values of Total Oxidised Nitrogen (TON) vary by up to a factor of 10. This can be largely attributed to the very high values encountered in the Burn 10 and Corrie catchments following clearfelling of a section of forest.

Decomposition of litter and brushwood combined with a reduced uptake by plant cover may account for most of the increase, but some may also be due to the practice of treating the tree stumps with urea to promote microbial breakdown. Between other catchments mean TON varies by less than a factor of three.

Mean Phosphate concentrations are around or below detectable limits in circumneutral waters and between 1 and 3 $\mu eq l^{-1}$ in most of the acidic burns, but rise to 66 $\mu eq l^{-1}$ (mean) for Burn 9 in Table 10, and 14 in Table 11. This is a consequence of the fertilization of the catchment carried out by the Forestry Commission. Phosphate will continue to be leached out of the soil for several years after such treatment (Harriman, 1978).

TABLE 10
MEAN VALUES OF ENVIRONMENTAL VARIABLES. MATRIX A

SITE	14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
pH inst.		4.68	5.89	5.19	5.20	5.71		4.30	4.38	4.39	4.39	4.33	6.03	6.20	6.48
pH comp.	4.95	4.70	-	5.12	5.18	5.98		4.33	4.47	4.47	4.37	4.37	-	6.19	-
'FLOW' m.		0.15*	0.19	0.33	0.33	0.43		0.17	0.17	0.15	0.17	0.18	-	0.15*	0.15
'DEPTH' m.	-	0.073*	-	0.135	0.143	0.061		0.129	0.106	0.108	0.130	0.138	-	0.10*	-
Temp °C	7.23	6.85	8.84	10.04	9.40			7.65	8.10	7.40	9.25	6.45	6.98	7.36	1.88
T max										10.50					4.55
T min										2.50					0.00
A_{400}		0.04	0.02	0.04	0.04	0.03		0.07	0.05	0.05	0.13	0.09	0.05	0.04	0.06
A_{350}	-	-	-	-	-	-		-	-	-	-	-	-	-	-
A_{250}		0.24	0.11	0.22	0.29	0.17		0.43	0.28	0.27	0.84	058	0.29	0.25	0.39
PAR%		90*		80*	80*	100*		80*	40*	90*	80*	50*		80*	
³PAR ABS															
# TON	11.18	11.18		5.12	5.47	7.00		4.24	16.41	16.41	3.82	3.82		27.41	
# PO_4	1.62	1.62		1.33		0.00		66.55	3.20	3.20	2.20	2.20		1.50	
²AL TM	138.90	138.90		61.00	79.93	44.53		177.67	236.65	236.65	172.88	172.88		57.06	
²Al NI	84.10	84.10		41.71	69.73	40.06		82.07	102.65	102.65	102.29	102.29		48.31	
²Al-L	54.80	54.80		19.29	10.20	4.47		98.00	134.00	134.00	70.59	70.59		8.75	
#ALK	9.09	9.09		14.06	12.06	36.47		0.00	0.00	0.00	0.00	0.00		129.59	
#Ca^{++}	72.82	72.82		52.47	66.82	73.76		56.65	62.76	62.76	53.88	3.88		175.88	
#Mg^{++}	47.64	47.64		37.00	38.29	49.94		58.35	65.59	65.59	48.88	48.88		138.12	
²SiO_2	1645.37	1645.37		548.00		1143.33		1791.78	1973.87	1973.87	1930.87	1930.87		2508.87	
A_{250} comp	0.25	0.25		0.19	0.26	0.15		0.44	0.23	0.23	0.50	0.50		0.24	
#H^+ inst		20.79	1.60	6.40	6.35	1.93		50.56	40.90	41.07	40.53	46.33	0.93	0.63	0.33
#H^+ comp	20.18	19.97		7.60	6.65	1.04		46.58	34.28	34.28	42.50	42.50		0.64	
	^		^				^						^		^

#micro-equivalents per litre
²micrograms per litre
³micromoles per m² per second (24 hour average)

*Values interpolated for analysis by CANOCO
ND = Not detectable

^ These S.U.s omitted for analysis by CANOCO

TABLE 11
MEAN VALUES OF ENVIRONMENTAL VARIABLES. MATRIX B

SITE	14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
pH inst.	5.39	4.77	6.09	5.31	5.30	5.87	5.41	4.46	4.58	4.57	4.45	4.42	6.25	6.46	6.68
pH comp.	5.29	5.05	6.00*	5.37	5.45	6.19	5.55*	4.47	4.63	4.63	4.53	4.53	6.20*	6.82	6.70*
'FLOW' m.	0.25	0.25	0.17	0.29	0.34	0.42	0.39*	0.14	0.11	0.13	0.15	0.15	0.15*	0.10*	0.16
'DEPTH' m.	0.078	0.073	0.157	0.135	0.143	0.061	0.100*	0.129	0.106	0.108	0.130	0.138	0.10*	0.10*	0.065
Temp °C	8.24	8.06	8.47	9.91	11.21	10.97	14.50	9.56	7.74	7.88	8.65	8.59	7.55	9.46	10.38
T max				13.30		13.92					13.82			9.80	12.90
T min				3.90		2.28					4.74			3.50	3.60
A_{400}	0.04	0.04	0.02	0.03	0.04	0.02	0.03	0.08	0.04	0.04	0.09	0.08	0.04	0.04	0.11
A_{350}	0.10	0.13	0.05	0.07	0.10	0.06	0.09	0.18	0.10	0.09	0.19	0.18	0.10	0.10	0.25
A_{250}	0.31	0.35	0.15	0.23	0.30	0.20	0.24	0.56	0.29	0.28	0.65	0.62	0.26	0.29	0.74
PAR%	60.6	90.0*	40.0*	81.5	78.4	100.0	70.0*	72.6	42.0	90.0*	68.5	50.0*	40.0*	57.4	90.0*
³PAR ABS	163.2			220.8	231.5	269.2		195.5	113.08					150.8	269.2
#TON	7.9	7.9	2.7	5.3	4.5	5.0	2.3	4.2	16.9	16.92	2.7	2.7	13.84	19.6	2.9
# PO_4	1.0	1.0	0.2	1.3	0.2	ND	0.1	13.7	1.7	1.7	2.3	2.3	0.2	1.5	1.3
²AL TM	111.2	111.2	54.0	40.8	71.2	37.5	72.0	127.6	203.6	203.6	146.9	146.9	52.0	33.9	102.5
²Al Nl	75.3	75.3	34.8	31.9	60.8	33.2	41.0	76.1	112.3	112.3	94.5	94.5	54.0	29.2	89.5
²Al-L	35.9	35.9	19.3	8.9	10.4	4.3	31.0	51.5	97.5	97.5	52.4	52.4	18.0	4.7	16.5
#ALK	12.8	12.8	50*	20.1	23.1	49.4	36.0*	0.6	0.0	0.0	0.0	0.0	50*	190.7	190*
# Ca^{++}	78.9	78.9	100*	52.8	65.4	74.8	70.0*	46.5	56.8	56.8	49.0	49.0	100*	196.5	190*
# Mg^{++}	45.5	45.5	50*	34.4	36.2	48.6	42.0*	45.7	56.5	56.5	41.7	41.7	50*	156.6	150*²
² SiO_2	1656	1656	1700*	1700*	1700*	1700*	1700*	1870	1836	1838	1925	1925	1700*	2894	2900*
A_{250} comp	0.25	0.25	-	0.20	0.29	0.18	-	0.52	0.32	0.32	0.53	0.53	-	0.29	-
# H^+ inst	10.78	17.12	1.23	4.85	5.01	1.35	3.91	34.62	30.20	29.5	35.9	37.8	0.57	0.35	0.21
# H^+ comp	9.00	8.94	-	4.23	3.59	0.65	-	33.86	25.46	25.5	29.4	29.4	-	0.15	-

#micro-equivalents per litre
²micrograms per litre
³micromoles per m^2 per second (24 hour average)

*Values interpolated for analysis by CANOCO
ND = Not detectable

TABLE 12

MEAN VALUES OF ENVIRONMENTAL VARIABLES. MATRIX C

SITE	14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
pH inst.	5.39	4.71	6.01	5.25	5.25	5.79	5.41	4.37	4.51	4.50	4.43	4.38	6.12	6.31	6.67
pH comp.	5.16	4.88	6.00*	5.22	5.29	6.08	5.50*	4.39	4.57	4.57	4.45	4.45	6.20*	6.41	6.69
FLOW	0.25	0.25	0.17	0.31	0.33	0.43	0.40*	0.15	0.14	0.14	0.16	0.17	0.15*	0.15*	0.16
DEPTH m	0.08	0.07	0.16	0.14	0.14	0.06	0.10*	0.13	0.11	0.11	0.13	0.14	0.10*	0.10*	0.07
Temp °C	8.24	7.43	7.87	9.38	10.68	10.23	14.50	8.68	7.91	7.68	8.84	7.80	7.28	8.66	9.49
T max				13.30		13.92				10.50	13.82			9.80	9.56
T min				3.90		2.28				2.50	4.74			3.50	2.16
A_{400}	0.04	0.04	0.02	0.03	0.04	0.03	0.03	0.07	0.04	0.04	0.11	0.09	0.04	0.04	0.10
A_{350}	0.10	0.13	0.05	0.07	0.10	0.06	0.09	0.18	0.10	0.09	0.19	0.18	0.10	0.10	0.25
A_{250}	0.31	0.28	0.14	0.23	0.30	0.18	0.24	0.50	0.29	0.28	0.71	0.60	0.28	0.27	0.73
PAR%	60.6	90*	40*	81.5	78.4	100.0	70.0*	72.6	42.0	90.0*	68.5	50.0*	40.0*	57.4	90*
[3]PAR ABS	163.2			220.8	231.5	269.2		195.5	113.1		184.4			150.8	269.2
# TON	9.1	9.1	2.7	5.2	5.0	6.0	2.3	4.2	16.6	16.6	3.3	3.3	13.8	23.4	2.9
# PO_4	1.2	1.2	0.2	1.3	0.2	ND	0.1	39.0	2.2	2.2	2.2	2.2	0.2	1.5	1.3
[2]AL TM	121.4	121.4	54.0	51.2	75.3	41.1	72.0	152.6	220.6	220.6	159.9	159.9	52.0	45.1	102.5
[2]Al NI	78.6	78.6	34.8	36.9	65.0	36.7	41.0	79.1	107.2	107.2	98.4	98.4	54.0	38.5	89.5
[2]Al-L	42.8	42.8	19.3	14.3	10.3	4.4	31.0	73.5	116.9	116.9	61.5	61.5	18.0	6.6	13.0
#ALK	11.4	11.4	50.0*	17.0	17.7	43.1	30.0*	0.3	0.0	0.0	0.0	0.0	50.0*	161.0	160.0*
#Ca^{++}	76.6	76.6	100.0*	52.6	66.1	74.3	70.0*	51.7	59.8	59.8	51.4	51.4	100.0*	186.5	190.0*
#Mg^{++}	46.3	46.3	50.0*	35.7	37.2	49.3	43.0*	52.2	61.1	61.1	45.2	45.2	50.0*	147.6	150.0*
[2]SiO_2	1653	1653	1700*	548	1700*	1143	1700*	1838	1897	1897	1927	1927	1700*	2276	1700*
A_{250} comp	0.25	0.25	-	0.19	0.27	0.17	-	0.48	0.28	0.28	0.52	0.52	-	0.27	-
#H^+ inst	10.8	19.4	1.4	5.6	5.6	1.6	3.9	42.4	35.4	35.4	37.6	41.8	0.76	0.48	0.22
#H^+ comp	13.2	13.1	-	6.0	5.1	0.8	-	40.4	29.7	29.7	35.8	35.8	-	0.39	-

#micro-equivalents per litre

[2]micrograms per litre

[3]micromoles per m^2 per second (24 hour average)

*Values interpolated for analysis by CANOCO

ND = Not detectable

Because of the bias introduced by these high values of PO_4^{3-} and TON it is unlikely that meaningful correlations between these variables and other variables (Table 13), biomass or species abundance data will be found. Nevertheless there appears to be a tendency for PO_4^{3-} to be higher in the more acidic waters (Table 12). This phenomenon has been noted previously (Nalewajko and O'Mahony, 1988) although the opposite effect is also reported (Jansson *et al*, 1986).

Concentrations of total monomeric aluminium (Al-TM) are highest in the most acidified burns 9, 10, 11 and 14. Some differences are found between these sites in the proportion present as labile aluminium (Al-L), thought to represent the more toxic form (Helliwell *et al*, 1983; Folsom *et al*, 1986).

These differences may be due to the different amounts of humic material present in these burns; Burn 10 for example has the highest proportion (as well as the highest actual amount) of Al-L, and the lowest A_{250} in this acidified group of burns. Aluminium can complex with humic material (Steinberg and Kuhnel, 1987; Gjessing *et al*, 1989). Aluminium also complexes with phosphate (Minzoni, 1984; Nalewajko and Paul, 1985) so that some of the >79 µg l^{-1} (approximately 9 µeq l^{-1}) of non-labile aluminium may be in the form of an alumino-phosphorus complex. The water was not filtered before analysis so that the proportion of phosphate present in such complexes cannot be determined. Acidification of water may cause the release of aluminium from particulates (Tipping *et al*, 1989) and will thus release phosphate at the same time. Therefore complexed phosphate is not necessarily unavailable to algae. Alkalinity and the divalent cations Ca^{2+} and Mg^{2+} behave as would be expected, being highest in the sites with the highest mean pH.

Silicate concentrations are positively correlated with pH due to the pH dependence of silicate solubility. A limited amount of data is available for SiO_2, so some interpolations have been made to enable this variable to be included in analysis by CANOCO. The biological significance of silicon results from its requirement by diatoms and its complexation reactions with aluminium, which can ameliorate its toxicity (Birchall *et al*, 1989).

Intercorrelations between environmental variables are of great importance in interpreting the influence of environment on species distributions. In Table 13 correlations between all pairs of variables is shown (Pearson's r), along with the number of paired observations (n) and the p-value. There are 171 separate correlations in the table, so the appropriate level of significance is 0.5% ($p = 0.005$).

Those correlations with $p < 0.001$ are most revealing about the relationships between the variables. The two measures of pH are strongly intercorrelated, but composite pH is usually found to give the higher correlation with other variables. pH is strongly correlated with

TABLE 13
INTERCORRELATIONS OF ENVIRONMENTAL VARIABLES
MATRIX C INDIVIDUAL SITE/SAMPLE DATA

		ANN. WEEK	pH inst	pH comp.	'FLOW'	'DEPTH'	TEMP	A_{400}	A_{350}	A_{250}	PAR	TON	PO_4	Al TM	Al NL	Al L	ALK.	Ca^{2+}	Mg^{2+}
pH inst	r	-0.001																	
	n	420																	
	p	0.899																	
pH comp	r	-0.013	0.960																
	n	311	286																
	p	0.867	<0.001																
'FLOW'	r	0.010	0.120	0.388															
	n	313	295	242															
	p	0.956	0.039	<0.001															
'DEPTH'	r	0.024	-0.395	-0.390	0.660														
	n	313	295	242	313														
	p	0.757	<0.001	<0.001	<0.001														
TEMP.	r	0.200	0.167	0.193	0.077	-0.087													
	n	390	382	269	286	286													
	p	<0.001	0.001	0.001	0.195	0.142													
A_{400}	r	0.225	-0.257	-0.358	-0.097	0.172	0.192												
	n	416	414	282	292	292	378												
	p	<0.001	<0.001	<0.001	0.097	0.003	<0.001												
A_{350}	r	0.199	-0.246	-0.522	0.054	0.341	0.179	0.999											
	n	151	151	93	111	111	139	151											
	p	0.024	0.002	<0.001	0.576	<0.001	0.035	<0.001											
A_{250}	r	0.159	-0.262	-0.374	-0.116	0.158	0.228	0.976	0.998										
	n	416	414	282	292	292	378	416	151										
	p	0.001	<0.001	<0.001	0.048	0.007	<0.001	<0.001	<0.001										
PAR	r	0.159	0.093	0.160	0.231	-0.111	0.628	-0.013	0.020	0.006									
	n	29	63	47	48	48	63	63	47	63									
	p	0.902	0.008	0.208	0.029	0.691	<0.001	0.946	0.806	0.867									
TON	r	-0.154	0.056	0.113	-0.053	0.094	-0.341	-0.206	-0.199	-0.226	-0.485								
	n	346	322	304	254	254	297	319	132	319	57								
	p	0.004	0.318	0.050	399	0.134	<0.001	<0.001	0.022	<0.001	<0.001								
PO_4	r	0.180	-0.222	-0.212	0.039	0.138	-0.004	0.139	0.259	0.095	-0.040	-0.084							
	n	168	163	126	119	119	149	161	96	161	36	168							
	p	0.020	0.004	0.017	0.675	0.135	0.965	0.079	0.011	0.232	0.642	0.279							
Al-TM	r	0.035	-0.790	-0.798	-0.384	0.233	-0.144	0.401	0.317	0.388	-0.188	0.144	0.286						
	n	307	282	295	234	234	265	279	97	279	51	302	132						
	p	0.582	<0.001	<0.001	<0.001	<0.001	0.019	<0.001	0.002	<0.001	0.002	0.012	0.001						
Al-NL	r	0.086	-0.658	-0.677	-0.282	0.242	-0.115	0.593	0.462	0.583	-0.283	0.103	0.148	0.846					
	n	304	279	293	232	232	262	276	94	276	51	299	131	304					
	p	0.107	<0.001	<0.001	<0.001	<0.001	0.064	<0.001	<0.001	<0.001	0.015	0.074	0.091	<0.001					
Al-L	r	-0.015	-0.715	-0.716	-0.385	0.165	-0.141	0.140	0.136	0.127	-0.400	0.152	0.322	0.894	0.540				
	n	304	279	293	232	232	262	276	94	276	51	299	131	304	304				
	p	0.660	<0.001	<0.001	<0.001	0.012	0.023	0.020	0.191	0.03	0.004	0.009	<0.001	<0.001	<0.001				
Alkalin.	r	0.023	0.713	0.779	0.408	-0.318	0.115	-0.215	-0.275	-0.218	-0.050	0.187	-0.114	-0.524	-0.499	-0.425			
	n	312	298	311	243	243	270	283	93	283	47	304	126	295	293	293			
	p	0.660	<0.001	<0.001	<0.001	<0.001	0.060	<0.001	0.001	<0.001	0.450	0.001	0.205	<0.001	<0.001	<0.001			
Ca^{2+}	r	0.051	0.673	0.728	0.059	-0.332	0.093	-0.160	-0.288	-0.176	-0.138	0.362	0.006	-0.382	-0.367	-0.307	0.915		
	n	310	285	309	241	241	268	282	93	282	47	304	126	295	293	293	310		
	p	0.336	<0.001	<0.001	0.360	<0.001	0.131	0.007	0.005	0.003	0.117	<0.001	0.950	<0.001	<0.001	<0.001	<0.001		
Mg^{2+}	r	0.058	0.481	0.520	-0.288	-0.131	0.018	-0.090	-0.195	-0.112	-0.217	0.447	0.058	-0.142	-0.203	-0.057	0.803	0.921	
	n	310	285	309	241	241	268	282	93	282	47	304	126	295	293	293	310	310	
	p	0.296	<0.001	<0.001	<0.001	0.043	0.772	0.132	0.061	0.061	0.012	<0.001	0.518	0.015	<0.001	0.327	<0.001	<0.001	
SiO_2	r	0.134	0.518	0.567	-0.569	-0.397	-0.136	-0.144	-0.439	-0.173	-0.169	0.296	-0.184	-0.372	-0.345	-0.324	0.536	0.608	0.630
	n	133	122	132	101	101	117	120	47	120	20	132	114	128	128	128	133	133	133
	p	0.120	<0.001	<0.001	<0.001	<0.001	0.143	0.117	0.002	0.059	0.059	0.620	0.050	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

r = Pearson correlation coefficient   n = number of paired observations   p = p value

alkalinity; Ca^{2+}, $Mg^{2+}$and SiO_2, and negatively with Aluminium. There is a weaker negative correlation with absorbance measures. Both pH inst. and pH comp. are negatively correlated with 'Depth', but show positive correlations, of which only pH comp. is significant, with 'Flow'. This latter is surprising since it is a well-recorded phenomenon that pH is reduced during storm-induced increases in discharge (R. Harriman, pers. comm.). It is likely that differences in the depth of the gauges in the stream bed at different sites may obscure this relationship in the data. When the correction of subtracting the lowest reading from the data is applied, to produce the 'Depth' variable, a significant negative correlation results, which is more in accordance with expectation.

All measures of absorbance are strongly intercorrelated. They are also positively correlated with total monomeric and non-labile aluminium, but not with labile aluminium, which confirms the complexation of labile Al with humic material (Steinberg and Kuhnel, 1987; Gjessing *et al*, 1989). A weaker negative correlation exists between absorbance and alkalinity and between TON and A_{400} or A_{250}. A weak correlation with annual week is found for A_{400}.

PAR is positively correlated with temperature, reflecting the causal relationship between them, and negatively correlated with TON. The latter could be due to nutrient uptake by terrestrial and aquatic plants during the growing season. The lack of any similar correlation with PO_4^{3-} seems surprising, but may be due to the presence of complexed PO_4^{3-} which is not taken up by plants, or due to a superfluity of phosphate in some waters.

TON is positively correlated with Ca^{2+}, Mg^{2+} and SiO_2, higher levels of which are associated with the less acidic waters. This correlation could be biased slightly by the clearfelling operation in the Corrie catchment but since a similar clearfell was carried out at Burn 10, it may reflect catchment differences in the rate of litter breakdown, due to the different soil types present.

PO_4^{3-} is positively correlated with Al TM and particularly with Al-L, but not with non-labile Al (Al-NL). This is surprising in view of the known complexation reactions of PO_4^{3-} with aluminium, (Minzoni, 1984; Nalewajko and Paul, 1985). However the extreme values of PO_4^{3-} in Burn 9 resulting from fertilization may be responsible for this discrepancy.

Intercorrelations between the different forms of aluminium are high. The lowest is between labile and non-labile Al, implying that other variables are involved, e.g. through complexation reactions, since there is not a simple proportionality relationship between these two forms.

Alkalinity being largely due to the availability of calcium and magnesium salts for neutralization reactions, is highly correlated with these two ions, and with pH. Due to the relationship between pH and silica solubility, this variable is also highly correlated with alkalinity, Ca^{2+} and Mg^{2+}.

3.3 BIOMASS

Mean values for biomass estimates in matrices A, B and C are shown in Tables 14 to 16. Very few values are available in Matrix A and consequently no trends are discernible. Matrix B and Matrix C values are thus almost identical since the majority of data in Matrix C are also present in Matrix B.

Using any of the biomass measures on either slides or tiles, the highest biomass appears to be developed in the highest-pH sites. The situation is less clear-cut with biomass on stones, where high values of pigments are found in sites 6, 9, 10-2, 11-2 and C-1 (carotenoid > 2 µspu cm^{-2}). The values at the remaining sites however are also high in comparison with values encountered on artificial substrates. This is presumably due to the sampling procedure used with stones, which involved selecting a stone with a dense growth and another with a low periphyton density. This presumably introduces bias in areal biomass estimates. The minimum values for stone biomass (mean values of the minimum in each sample) shown in Table 16 are larger than the mean values on slides and tiles for most sites.

The relationship between environmental conditions and biomass is investigated further through correlation analysis (Tables 17, 18), using Matrix C site-mean data. Only pH, 'Depth', alkalinity, calcium and H^+ show correlations significant at the 1% significance level. Al also shows correlations significant at the 5% level with AFDW on slides. All biomass measures on slides and pigments on tiles show high correlations with pH and 'Depth'. Pigment extracted from stones is not significantly correlated with any variable. Pigments are negatively correlated with 'Depth' which may be interpreted as meaning that where 'Depth' (= mean fluctuation in depth) is high, biomass may fail to accumulate, or may be more regularly washed away.

The highest correlations, of slide and tile pigments and slide AFDW with pH are positive suggesting that there is a decrease in biomass with a decrease in mean pH of a stream.Whether this relationship also applies where environmental variables fluctuate within each stream is investigated by calculating the correlations between variables and biomass determined in each sample (Table 19). The correlations obtained are lower but because of the larger number of observation pairs in most cases, some are nevertheless significant at or below the 1% level ($0.01<p<0.001$).

The significant negative correlation of slide AFDW with annual week number implies a decline in biomass with time, and must be a consequence of particular environmental conditions occurring during the sampling programme, for example a large biomass accumulated early in the year being removed by spates later in the season rather than a true seasonality which would result in a non-linear relationship. There is a smaller, but significant positive correlation between stone chlorophyll-*a* and annual week. This may be spurious considering

the poor correlations found between stone biomass and other variables, and the low intercorrelations existing between stone biomass and that on other substrates (Table 20).

Both instantaneous and composite pH are significantly positively correlated with slide and tile pigments, at the 1% significance level or lower. Some significant correlations betwen slide pigments and 'Flow', alkalinity, Ca^{2+}, Mg^{2+} and silicate also occur. The largest correlations occur between tile pigments and silicate ($r > 0.6$). While it is tempting to see this as evidence that the growth of diatoms is enhanced by higher levels of silicate, the correlations could be spurious since silicate has a correlation of 0.569 with composite pH. (The solubility of silicate is greater at higher pH). However the correlations between SiO_2 and slide pigments are lower, despite their high correlation with pH. There should be no difference between tile- and slide-derived biomass estimates in theory, and the results obtained suggest that extraneous or uncontrolled factors may be involved in the observed behaviour.

For example, the estimates of pigments from slides may be biased against some sites where abrasion by small stones was a particular problem, e.g. in sites 5, 9, 10-1, 10-2 and C-1.However, amongst the different biomass estimates (with the exception of chlorophyll-a and carotenoid estimates from the same sample) the highest correlations found are between tile and slide pigments.

It would appear from these results that while any extrapolation to a whole-stream estimate of areal biomass would be unsafe, the evidence is not in favour of the contention that an increase of biomass occurs upon acidification. This is in agreement with the reports of Stokes (1981) and Marker and Willoughby (1988). It contrasts with the reports of Hendrey (1976), Muller (1980) and many anecdotal accounts of the condition of acidified streams which may reflect the greater visual impact of filamentous green algae than of an equal biomass of diatoms or blue-green algae. Hendrey's (1976) findings of a higher biomass but lower productivity (by ^{14}C incorporation) were interpreted as evidence for lower heterotrophic activity with decreased pH. However his results were obtained in artificial stream channels rather than in natural streams subjected to fluctuating flows, and measurement was discontinued when sloughing of algae occurred. Muller's (1980) studies were of periphytic algae in enclosures of natural lakes, artificially acidified, where removal by current or abrasion would be minimal. Therefore these two reports are not necessarily at odds with field studies suggesting no negative correlation between pH and biomass.

The issue of whether biomass developed at different pH values is different in artificial conditions has been addressed also (see results of culture experiments in Sections 3.4.4 and 3.5).

TABLE 14
MEAN VALUES (S.D.) OF BIOMASS MEASURES ON DIFFERENT SUBSTRATES
MATRIX A

	SLIDES			TILES			STONES	
SITES	AFDW mg.cm^{-2}	Chl-a µg.cm^{-2}	Carot. µspu.cm^{-2}	AFDW mg.cm^{-2}	Chl-a µg.cm^{-2}	Carot. µspu.cm^{-2}	Chl-a µg.cm^{-2}	Carot µspu.cm^{-2}
14-1	-	-	-	-	-	-	-	-
14-2	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-
2	-	0.37 (0.39)	-	-	0.17 (*)	-	-	-
5	-	0.63 (*)	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
5/6	-	-	-	-	-	-	-	-
9	0.39 (*)	2.05 (*)	-	-	0.30 (0.41)	-	-	-
10-1	-	0.76 (0.39)	-	-	0.08 (0.11)	-	-	-
10-2	0.49 (*)	0.89 (1.05)	-	-	-	-	-	-
11-1	0.13 (*)	1.32 (*)	-	-	0.64 (*)	-	-	-
11-2	-	0.31 (0.13)	-	-	-	-	-	-
C-1	-	-	-	-	-	-	-	-
C-2	0.48 (*)	2.59 (0.30)	-	-	1.26 (*)	-	-	-
Ward	-	-	-	-	-	-	-	-

* only one value available

TABLE 15
MEAN VALUES (S.D.) OF BIOMASS MEASURES ON DIFFERENT SUBSTRATES
MATRIX B

	SLIDES			TILES			STONES	
	AFDW mg.cm^{-2}	Chl-a µg.cm^{-2}	Carot. µspu.cm^{-2}	AFDW mg.cm^{-2}	Chl-a µg.cm^{-2}	Carot. µspu.cm^{-2}	Chl-a µg.cm^{-2}	Carot µspu.cm^{-2}
SITES								
14-1	0.69 (0.85)	1.94 (2.17)	0.90 (0.83)	0.18 (0.23)	0.63 (0.63)	0.33 (0.23)	3.04 (1.77)	1.31 (0.68)
14-2	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	4.51 (1.30)	1.44 (0.48)
2	0.78 (1.13)	0.86 (0.63)	0.34 (0.20)	0.15 (0.22)	0.23 (0.23)	0.10 (0.09)	1.45 (0.72)	0.67 (0.28)
5	0.89 (1.63)	1.03 (0.64)	0.37 (0.23)	0.15 (0.17)	0.68 (0.32)	0.29 (0.12)	3.24 (2.00)	1.22 (0.57)
6	2.15 (2.53)	5.27 (3.38)	1.59 (0.76)	0.69 (0.86)	2.88 (2.47)	0.91 (0.63)	5.85 (3.96)	2.22 (1.43)
5/6	-	-	-	-	-	-	-	-
9	0.44 (0.37)	0.71 (0.59)	0.28 (0.30)	3.28 (5.38)	0.54 (0.56)	0.31 (0.27)	5.47 (4.09)	2.20 (1.41)
10-1	0.58 (0.82)	0.89 (0.70)	0.39 (0.41)	0.20 (*)	0.65 (0.75)	0.39 (0.39)	2.92 (2.34)	1.19 (0.75)
10-2	0.73 (1.15)	1.02 (0.76)	0.42 (0.39)	-	0.11 (*)	0.05 (*)	4.92 (2.57)	2.25 (0.94)
11-1	0.80 (1.34)	1.63 (1.54)	0.51 (0.56)	0.25 (*)	0.44 (0.38)	0.24 (0.29)	3.65 (2.70)	1.42 (0.70)
11-2	0.08 (*)	0.26 (0.15)	-	-	0.32 (*)	0.12 (*)	5.07 (3.09)	2.43 (1.26)
C-1	-	-	-	-	-	-	5.93 (2.77)	2.65 (0.87)
C-2	2.57 (3.85)	3.46 (2.64)	1.67 (1.44)	0.27 (0.25)	1.47 (1.29)	0.60 (0.45)	4.16 (2.32)	1.71 (0.63)
Ward	1.91 (3.06)	3.10 (2.00)	1.19 (0.60)	0.70 (0.75)	2.88 (2.66)	1.26 (1.13)	4.05 (2.14)	1.33 (0.65)

* only one value available

TABLE 16
MEAN VALUES (S.D.) OF BIOMASS MEASURES ON DIFFERENT SUBSTRATES
MATRIX C

	SLIDES			TILES			STONES		STONES: MEAN VALUES OF MINIMA	
	AFDW mg.cm^{-2}	Chl-a µg.cm^{-2}	Carot. µspu.cm^{-2}	AFDW mg.cm^{-2}	Chl-a µg.cm^{-2}	Carot. µspu.cm^{-2}	Chl-a µg.cm^{-2}	Carot. µspu.cm^{-2}	Chl-a µg.cm^{-2}	Carot. µspu.cm^{-2}
SITES										
14-1	0.69(0.85)	1.94(2.17)	0.90(0.83)	0.18(0.23)	0.63(0.63)	0.44(0.23)	3.04(1.77)	1.31(0.68)	2.32(1.47)	0.97(0.55)
14-2	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	4.51(1.30)	1.44(0.48)	2.41(1.32)	0.87(0.55)
2	0.78(1.13)	0.79(0.62)	0.34(0.20)	0.15(0.22)	0.23(0.22)	0.10(0.09)	1.45(0.72)	0.67(0.28)	0.79(0.50)	0.33(0.23)
5	0.89(1.63)	0.98(0.61)	0.37(0.23)	0.15(0.17)	0.68(0.32)	0.29(0.12)	3.24(2.00)	1.22(0.57)	2.60(1.91)	1.07(0.45)
6	2.15(2.53)	5.27(3.38)	1.59(0.76)	0.68(0.86)	2.88(2.47)	0.91(0.63)	5.85(3.96)	2.22(1.43)	3.51(3.69)	1.39(1.22)
5/6	-	-	-	-	-	-	-	-	-	-
9	0.43(0.34)	0.88(0.72)	0.28(0.30)	3.28(5.38)	0.51(0.54)	0.31(0.27)	5.47(4.09)	2.20(1.41)	2.20(1.50)	1.02(0.70)
10-1	0.58(0.82)	0.86(0.63)	0.39(0.41)	0.20(*)	0.54(0.71)	0.39(0.39)	2.92(2.34)	1.19(0.75)	2.01(1.43)	0.89(0.60)
10-2	0.70(1.08)	0.98(0.79)	0.42(0.39)	-	0.11(*)	0.05(*)	4.92(2.57)	2.25(0.94)	4.07(2.38)	1.88(0.91)
11-1	0.74(1.29)	1.60(1.45)	0.51(0.56)	0.25(*)	0.46(0.37)	0.24(0.29)	3.65(2.70)	1.42(0.70)	3.24(2.33)	1.27(0.56)
11-2	0.08 (*)	0.31(0.13)	-	-	0.32(*)	0.12(*)	5.07(3.09)	2.43(1.26)	3.36(1.64)	1.45(0.67)
C-1	-	-	-	-	-	-	5.93(2.77)	2.65(0.87)	3.99(2.63)	1.74(0.80)
C-2	2.40(3.72)	3.29(2.37)	1.67(1.44)	0.27(0.25)	1.45(1.24)	0.60(0.45)	4.16(2.32)	1.71(0.64)	3.26(2.07)	1.26(0.43)
Ward	1.91(3.06)	3.10(2.00)	1.19(0.60)	0.70(0.75)	2.88(2.66)	1.26(1.13)	4.05(2.14)	1.33(0.65)	3.08(1.76)	0.93(0.47)

* only one value available

TABLE 17
CORRELATIONS OF BIOMASS WITH ENVIRONMENTAL VARIABLES
SITE-AVERAGE VALUES, MATRIX C

		SLIDE			TILE			STONE	
		A.F.D.W.	Chl.a	Carot.	A.F.D.W.	Chl.a	Carot.	Chl.a	Carot.
pH ins.	r	0.876	0.729	0.810	0.194	0.798	0.803	0.095	-0.092
	n	11	11	10	9	11	11	13	13
	p	<0.001	0.001	0.004	0.422	0.003	0.003	0.758	0.765
pH comp.	r	0.920	0.782	0.844	0.300	0.846	0.831	0.017	-0.187
	n	11	11	10	9	11	11	12	12
	p	<0.001	0.004	0.002	0.464	<0.001	<0.001	0.960	0.581
'Depth'	r	-0.762	-0.824	-0.897	-0.537	-0.788	-0.782	-0.173	-0.113
	n	10	10	9	8	10	10	11	11
	p	0.010	0.003	<0.001	0.796	0.007	0.008	0.611	0.741
P.A.R.	r	0.364	0.446	0.216	0.488	0.621	0.510	0.304	0.155
	n	11	11	10	9	11	11	13	13
	p	0.206	0.141	0.624	0.700	0.128	0.267	0.944	0.779
Al-TM	r	-0.664	-0.587	-0.583	-0.394	-0.526	-0.418	-0.036	0.100
	n	11	11	10	9	11	11	13	13
	p	0.026	0.058	0.077	0.573	0.096	0.201	0.907	0.745
Al-NL	r	-0.603	-0.533	-0.506	-0.451	-0..371	-0.238	-0.021	0.105
	n	11	11	10	9	11	11	13	13
	p	0.049	0.091	0.135	0.793	0.262	0.482	0.947	0.734
Al-L	r	-0.640	-0.568	-0.583	-0.317	-0.573	-0.486	0.011	0.155
	n	11	11	10	9	11	11	13	13
	p	0.034	0.068	0.077	0.481	0.066	0.129	0.973	0.613
Alkalin	r	0.846	0.573	0.793	0.078	0.504	0.545	0.072	0.012
	n	10	10	9	8	10	10	10	10
	p	<0.001	0.084	0.011	0.641	0.138	0.103	0.844	0.973
Ca^{2+}	r	0.778	0.503	0.761	-0.038	0.412	0.486	0.059	0.018
	n	10	10	9	8	10	10	10	10
	p	0.008	0.139	0.017	0.614	0.237	0.154	0.872	0.961
Mg^{2+}	r	0.657	0.368	0.631	-0.114	0.276	0.375	0.159	0.147
	n	10	10	9	8	10	10	10	10
	p	0.039	0.296	0.068	0.873	0.440	0.285	0.661	0.686
H^+	r	-0.772	-0.646	-0.667	-0.271	-0.636	-0.587	0.091	0.244
	n	11	11	10	9	11	11	13	13
	p	0.005	0.032	0.035	0.182	0.035	0.058	0.768	0.421
H^+ comp.	r	-0.752	-0.610	-0.638	-0.356	-0.587	-0.533	0.277	0.372
	n	11	11	10	9	11	11	13	13
	p	0.012	0.061	0.064	0.163	0.074	0.113	0.439	0.290

TABLE 18
INTERCORRELATIONS OF BIOMASS VALUES
SITE AVERAGE VALUES, MATRIX C

		SLIDE			TILE			STONE
		A.F.D.W.	Chl.a	Carot.	A.F.D.W.	Chl.a	Carot.	Chl.a
Slide Chl.a	r	0.892						
	n	11						
	p	0.001						
Slide Carot.	r	0.940	0.929					
	n	10	10					
	p	<0.001	<0.001					
Tile AFDW	r	0.472	0.775	0.520				
	n	9	9	9				
	p	0.568	0.765	0.579				
Tile Chl.a	r	0.830	0.890	0.803	0.625			
	n	11	11	10	9			
	p	0.002	<0.001	0.005	0.962			
Tile Carot.	r	0.780	0.792	0.733	0.422	0.966		
	n	11	11	10	9	11		
	p	0.226	<0.001	<0.001	0.037	<0.001		
Stone Chl.a	r	0.181	0.369	0.378	0.615	0.354	0.259	
	n	11	11	10	9	11	11	
	p	0.594	0.264	0.0281	0.081	0.286	0.441	
Stone Carot.	r	-0.031	0.149	0.268	0.594	0.090	-0.019	0.932
	n	11	11	10	9	11	11	13
	p	0.928	0.661	0.454	0.060	0.793	0.956	<0.001

TABLE 19
CORRELATIONS OF BIOMASS WITH ENVIRONMENTAL VARIABLES
INDIVIDUAL SITE/SAMPLE VALUES, MATRIX C

		SLIDE			TILE			STONE	
		A.F.D.W.	Chl.a	Carot.	A.F.D.W.	Chl.a	Carot.	Chl.a	Carot.
Annual Week	r	-0.585	0.202	0.177	-0.293	0.251	0.175	0.266	0.246
	n	97	113	65	28	105	55	361	80
	p	<0.001	0.029	0.166	0.130	0.009	0.214	<0.001	0.020
pH inst.	r	0.274	0.369	0.353	0.307	0.394	0.350	-0.032	-0.026
	n	94	110	65	28	105	55	163	80
	p	0.007	<0.001	0.004	0.112	<0.001	0.009	0.684	0.816
pH comp.	r	0.319	0.448	0.473	0.187	0.416	0.410	-0.022	-0.055
	n	74	88	50	21	81	43	120	59
	p	0.006	<0.001	0.001	0.418	<0.001	0.006	0.810	0.678
'Flow'	r	0.228	0.446	0.416	0.240	0.299	0.279	-0.031	-0.141
	n	78	90	50	22	84	39	130	61
	p	0.044	<0.001	0.003	0.282	0.006	0.085	0.729	0.277
Alkalinity	r	0.281	0.391	0.458	0.064	0.276	0.301	-0.027	0.059
	n	74	88	50	21	81	43	120	59
	p	0.015	<0.001	0.001	0.783	0.013	0.050	0.771	0.656
Ca^{2+}	r	0.288	0.324	0.444	-0.009	0.222	0.291	0.011	0.046
	n	74	88	50	21	81	43	119	59
	p	0.013	0.002	0.001	0.971	0.046	0.059	0.902	0.729
Mg^{2+}	r	0.254	0.293	0.450	-0.041	0.179	0.256	0.008	0.058
	n	74	88	50	21	81	43	119	59
	p	0.029	0.006	0.001	0.859	0.109	0.098	0.935	0.662
SiO_2	r	0.108	0.457	0.398	0.048	0.612	0.636	-0.068	-0.150
	n	32	35	19	8	42	21	54	31
	p	0.557	0.006	0.092	0.910	<0.001	0.002	0.623	0.420

TABLE 20
INTERCORRELATIONS OF BIOMASS VALUES
INDIVIDUAL SITE/SAMPLE VALUES, MATRIX C

		SLIDE			TILE			STONE
		A.F.D.W.	Chl.a	Carot.	A.F.D.W.	Chl.a	Carot.	Chl.a
Slide Chl.a	r	0.227						
	n	97						
	p	0.025						
Slide Carot.	r	0.244	0.954					
	n	65	65					
	p	0.050	<0.001					
Tile AFDW	r	0.334	0.508	0.260				
	n	22	23	13				
	p	0.129	0.013	0.390				
Tile Chl.a	r	0.094	0.668	0.591	0.596			
	n	69	72	51	27			
	p	0.452	<0.001	<0.001	0.001			
Tile Carot.	r	0.184	0.614	0.596	0.582	0.962		
	n	45	45	45	13	55		
	p	0.226	< 0.001	< 0.001	0.037	<0.001		
Stone Chl.a	r	-0.008	0.326	0.354	-0.188	0.176	0.152	
	n	83	89	58	27	81	49	
	p	0.943	0.002	0.006	0.347	0.118	0.298	
Stone Carot.	r	-0.077	0.319	0.350	-0.080	-0.040	0.024	0.955
	n	42	42	42	4	41	41	80
	p	0.629	0.039	0.023	0.920	0.802	0.881	<0.001

3.4 CHANNEL GROWTH EXPERIMENTS

3.4.1 Effect of pH on growth rate

Measurements of cell number were made at intervals of 2-4 days, and the growth rate between measurements calculated. The mean growth rates of various species at different pH values is shown in Figs. 3 to 12. Different growth rates were obtained in different experimental runs, possibly due to differences in the physiological condition of the inocula. Differences were also found in the growth rates during successive time periods, some species clearly showing a progressive decrease in growth and eventual death under certain conditions.

The individual species figures show the growth at each pH in sequential time periods.

Hormidium subtile and *Geminella* 8µm. show little effect of pH, and no diminution of growth rate with time. The growth rate of *Hormidium* is around 0.4 divisions per day (d/d), and *Geminella* approximately 0.3 to 0.7 d/d.

Stigeoclonium 5µm appears to have a maximum growth rate around pH 5.5, while the evidence concerning changes in growth rate with time is somewhat uncertain.

Stigeoclonium 8µm and *Draparnaldia* sp. fail to grow at pH 4.0. *Stigeoclonium* 8µm has an apparent growth maximum at pH 5.0 ,while *Draparnaldia* has a maximum at pH 6.0 or above, although the growth rate appears to diminish with time especially at pH 4.5.

Oedogonium species appear to have a growth optimum between pH 4.5 and 5.5 but at all values the growth rate decreases significantly with time, especially at pH 4.0. *Oedogonium* could not be maintained in long-term culture.

Four species of *Mougeotia* display broadly similar patterns of growth, with a maximum between pH 4.5 and 5.5. There is probably no diminution with time.

Comparing the mean growth rates of different species at each pH (Figs. 14-16) at pH 4.0-4.5, the highest growth rates are shown by *Stigeoclonium* 5µm. and *Hormidium* , with *Geminella* outgrowing *Hormidium* as pH rises. *Oedogonium* 15µm. has a growth maximum at pH 4.5, but this was only achieved in the first time period (fig. 13).

At a pH of 5.0-5.5, *Oedogonium* 6µm., *Mougeotia* 8µm and *Stigeoclonium* 8µm also achieve a growth rate of approximately 0.4 divisions/day. At pH 5.5 to 6.0 most of the above species show a decline in growth rate. Only *Draparnaldia* may show a continued increase in growth rate with pH, but it, along with *Mougeotia* 22 µm. did not achieve a growth rate greater than 0.3 d/d.

If these growth rates were to occur in natural populations, at pH values below 4.5, the community would be dominated by *Stigeoclonium* 5µm and *Hormidium*, with *Geminella* possibly replacing *Stigeoclonium* at a slightly higher pH. Other environmental factors may play a role in determining which taxon dominates; strength of filament and attachment to the substrate may influence dominance in a period of fluctuating current regime, for example.

Table 21a-f shows the results of analysis of variance of growth rates (ANOVA in MINITAB). The full model has 12 species, 5 pH treatments, 4 time periods and three replicate experiments, but there are numerous empty cells so that analysis of this model could not be carried out. Instead it was broken down by omitting species, time periods or replicates (but not treatments) in 6 different combinations.

The effect of species is significant at the 0.1% level in all cases. In 4 analyses treatment is significant at the 1.0% level, in one at the 10% level, but in the last is not significant. This analysis contains 4 species which are common in samples from acidic sites; *Stigeoclonium* 5µm, *Geminella* 5µm, *Hormidium subtile*, and *Mougeotia* 5µm. Time period is significant in some analyses but not in others, again depending upon the species involved in the analysis. Replicate was only subject to analysis in two cases, and had a significant effect at the 10% level in one case and the 0.1% level in the other. All analyses of variance fulfilled the requirement of normally distributed residuals.

There is clearly an inability of certain species to grow at low pH, while some of the species common in acidified streams appear to have a growth maximum at pH values between 4.5 and 5.5, confirming that they are acidobiontic.

The results of chemical measurements carried out on the media are shown in Table 22. In field samples low pH is usually associated with elevated levels of monomeric aluminium, though the proportion of labile monomeric Al may be low in highly humic water (Driscoll, 1984). Therefore the presence of 'acidobiontic' species may be a response to Al rather than pH.

FIG.3:

GROWTH RATE DURING SUCCESSIVE PERIODS OF EXPOSURE TO MEDIA OF DIFFERENT pH, OF *Hormidium subtile*

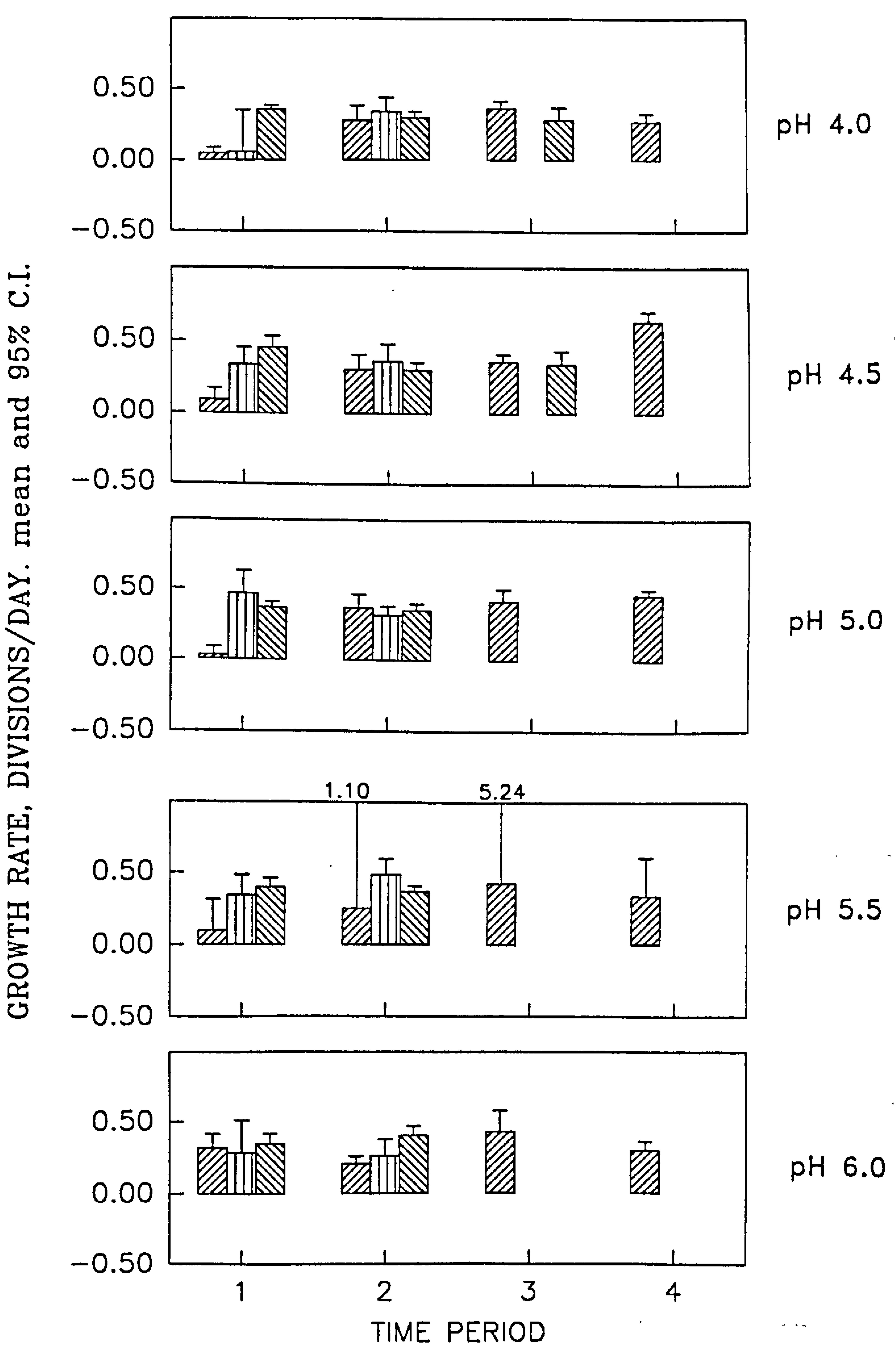


FIG.4:

GROWTH RATE DURING SEQUENTIAL PERIODS OF EXPOSURE TO MEDIA OF DIFFERING pH, OF *Geminella* 8μm.

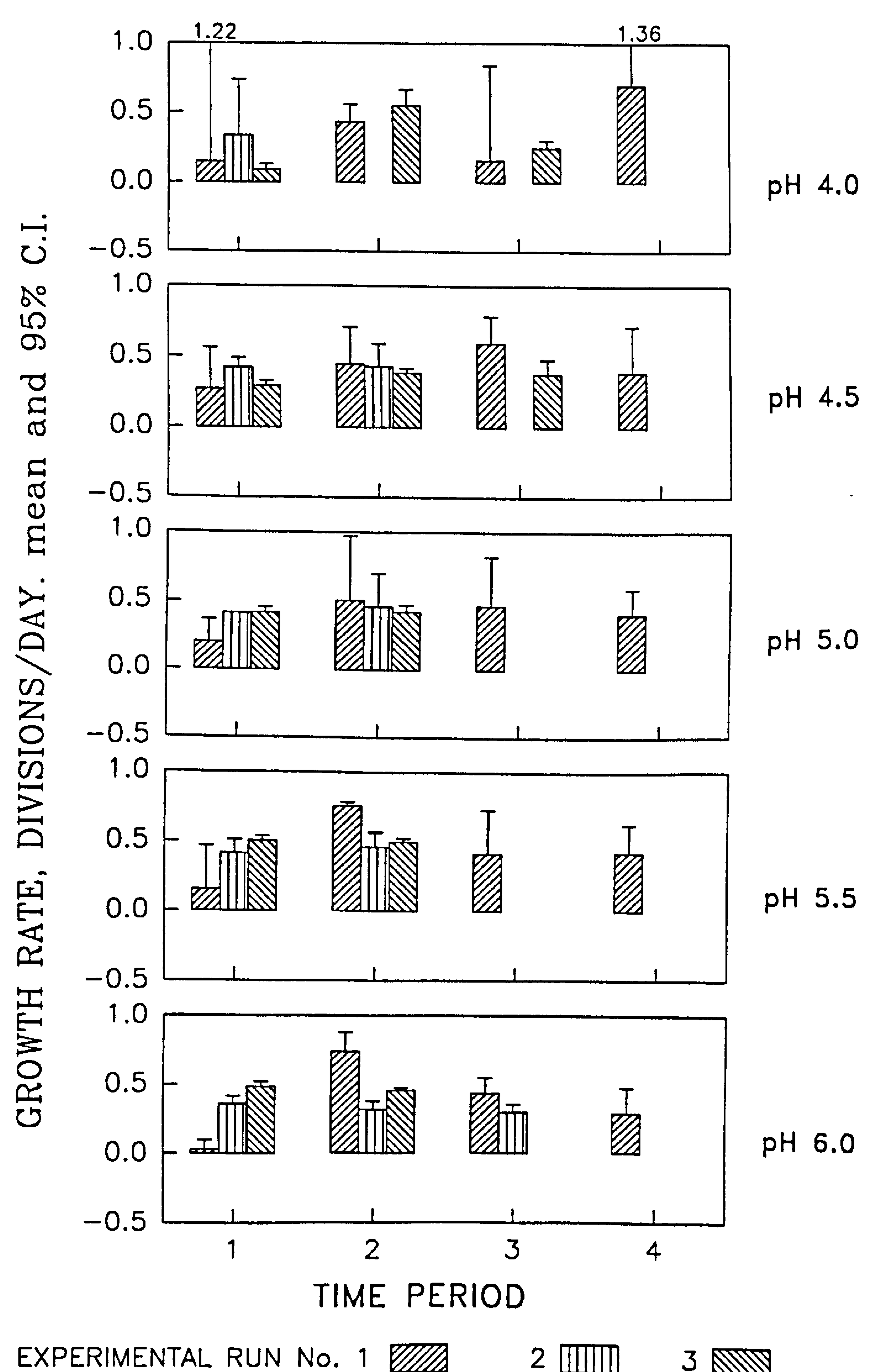


FIG.5 :

GROWTH RATE DURING SEQUENTIAL PERIODS OF EXPOSURE TO MEDIA OF DIFFERING pH, OF *Stigeoclonium* 5μm.

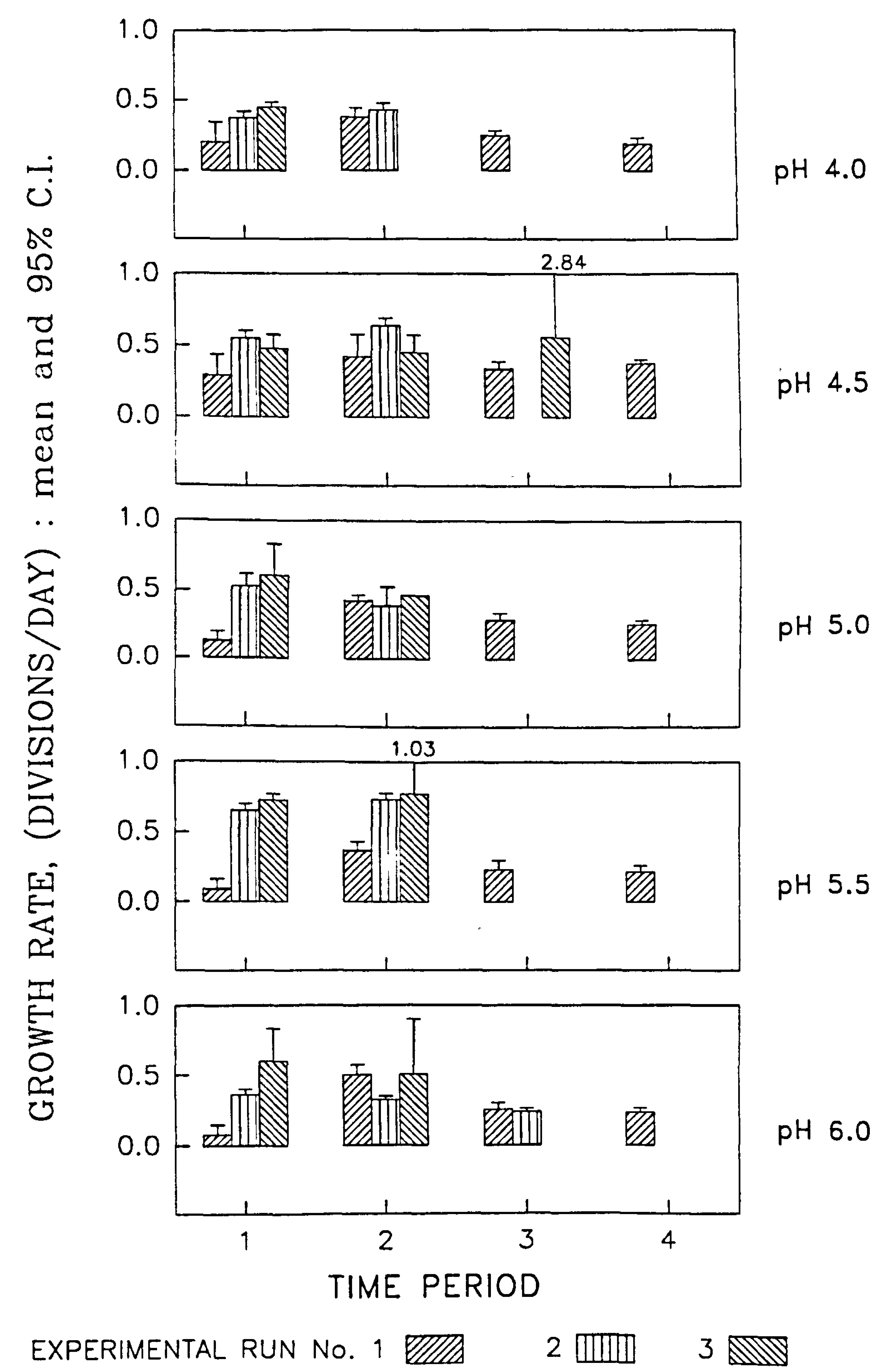
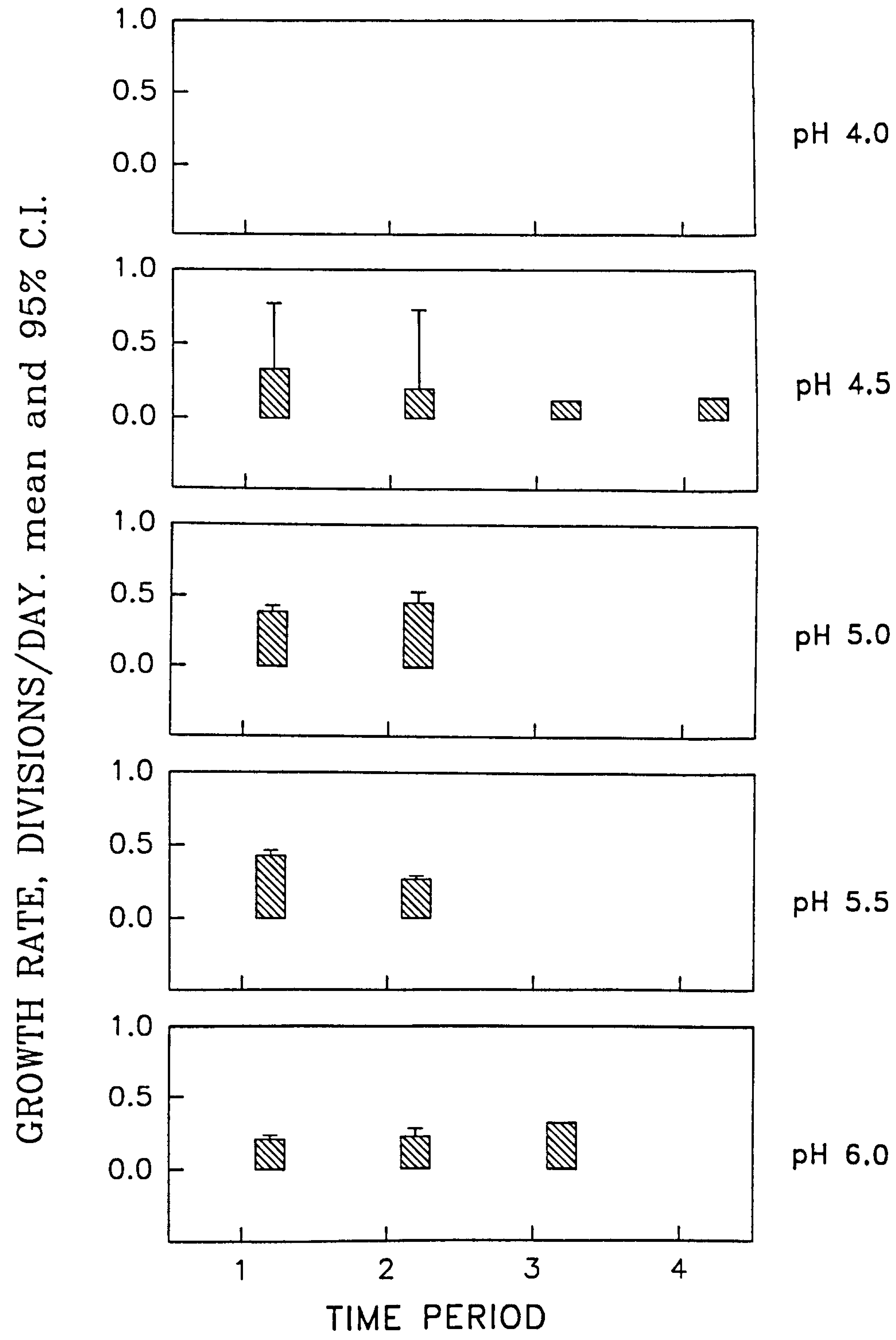


FIG. 6:

GROWTH RATE DURING SEQUENTIAL PERIODS OF EXPOSURE TO MEDIA OF DIFFERING pH, OF *Stigeoclonium sp.* 8μm.



(Lack of error bars indicates only one measurement available)

FIG. 7

GROWTH RATE DURING SEQUENTIAL PERIODS OF EXPOSURE TO MEDIA OF DIFFERING pH, OF *Draparnaldia sp.*

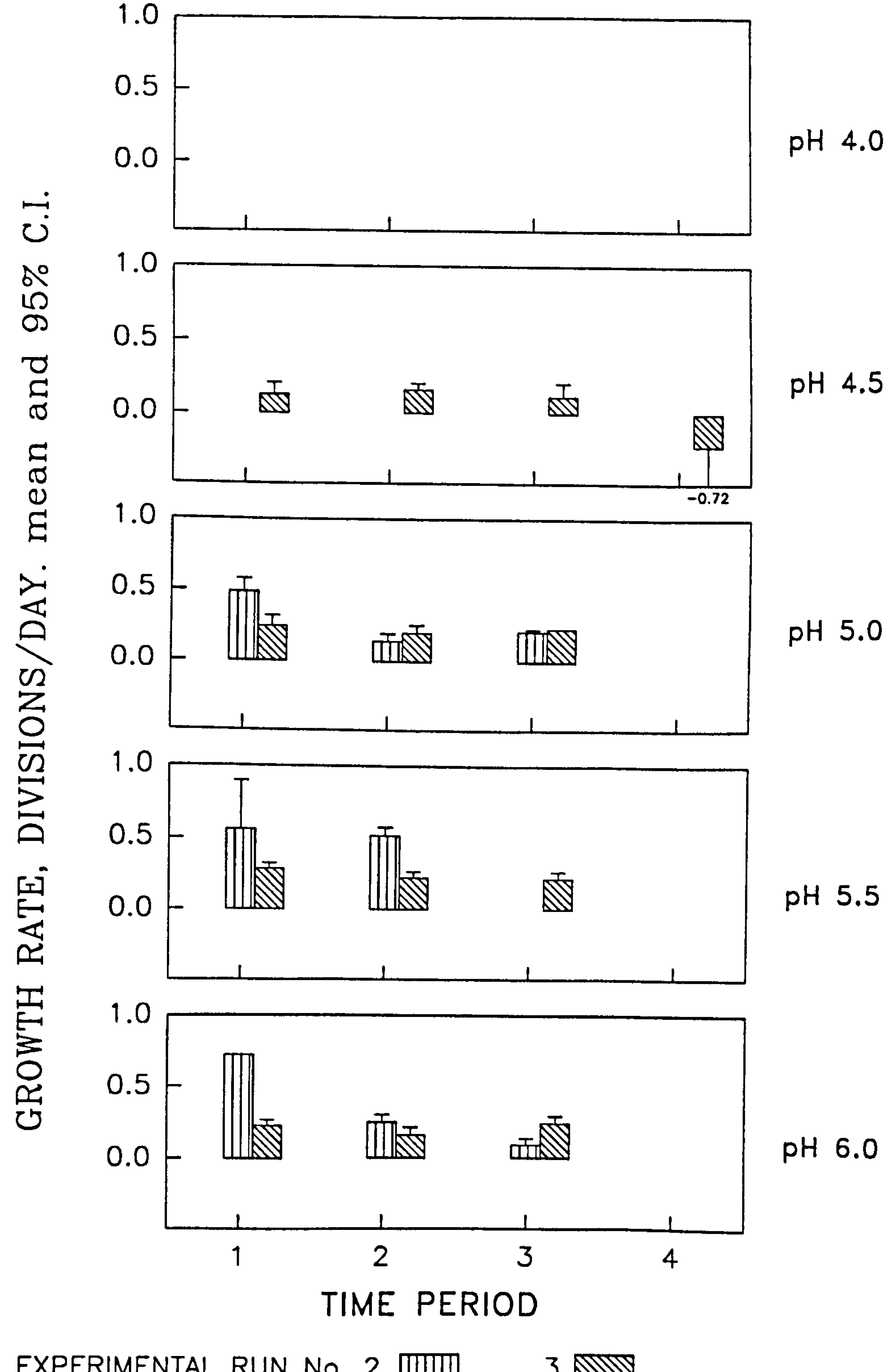
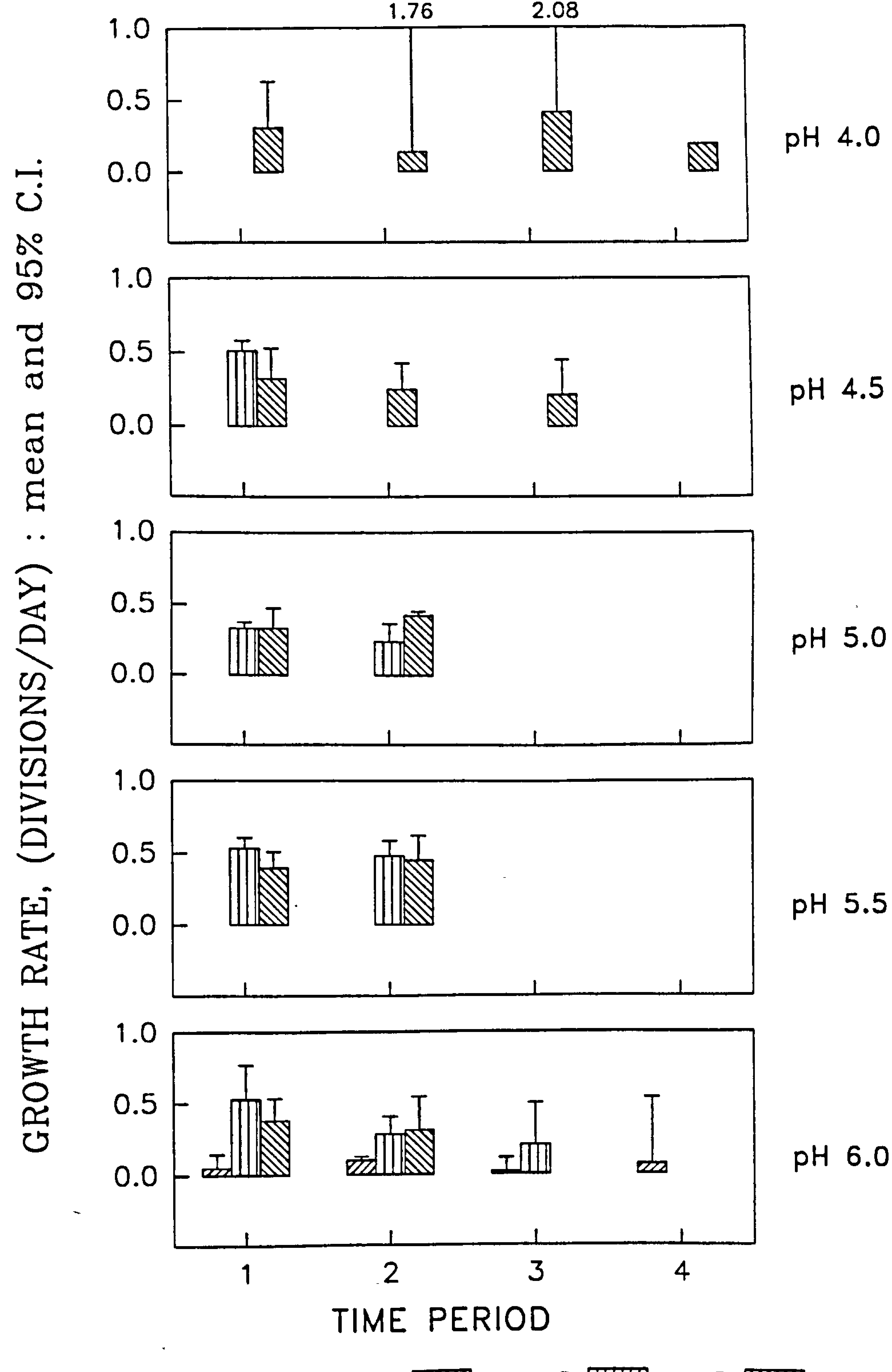


FIG.8 :
GROWTH RATE DURING SEQUENTIAL PERIODS OF EXPOSURE TO MEDIA OF DIFFERING pH, OF *Mougeotia* 8μm.



(Lack of error bars indicates only one measurement available)

FIG.9:

GROWTH RATE DURING SEQUENTIAL PERIODS OF EXPOSURE TO MEDIA OF DIFFERING pH, OF *Mougeotia* 11 μm.

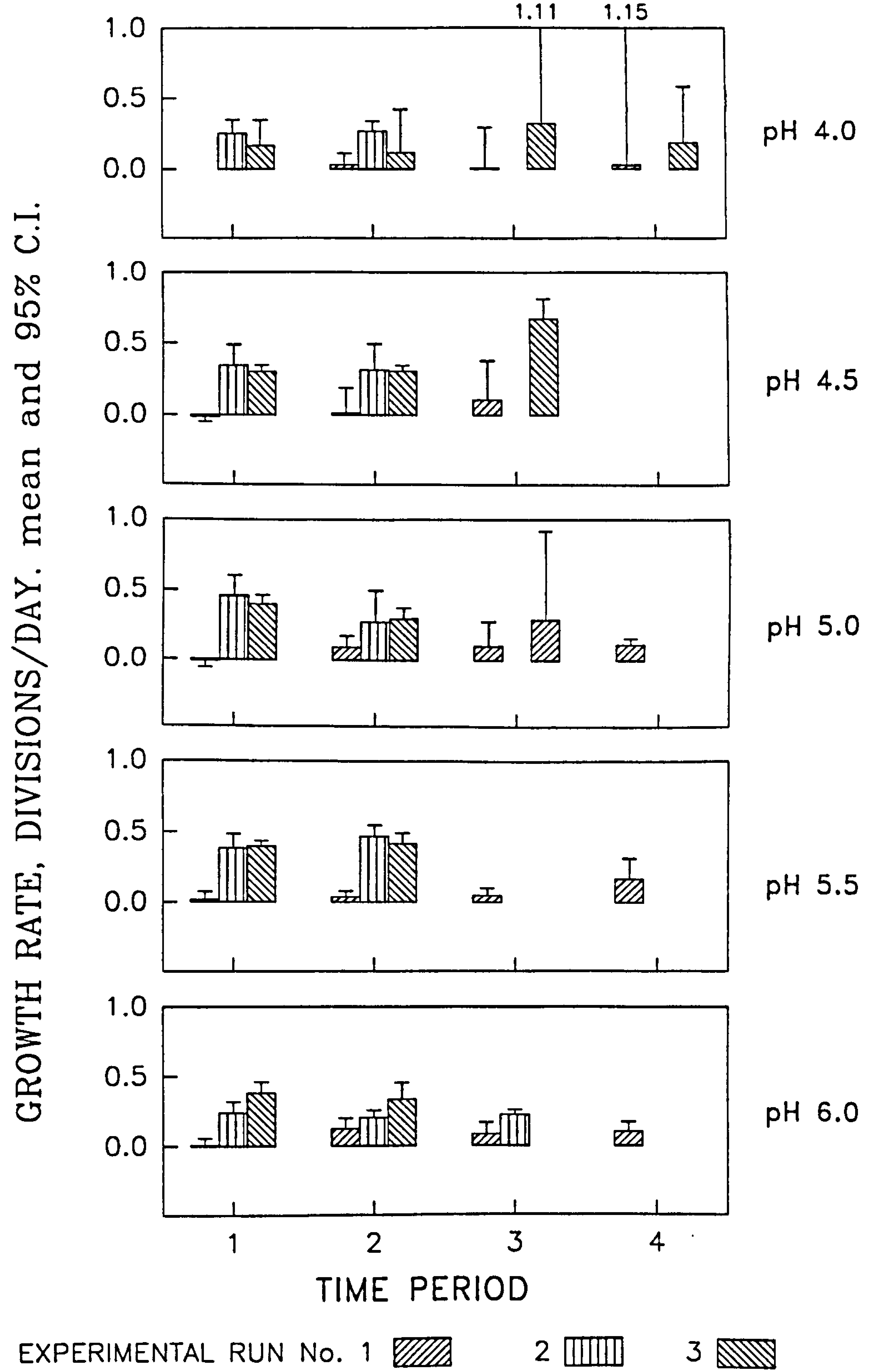


FIG.10 :

GROWTH RATE DURING SEQUENTIAL PERIODS OF EXPOSURE TO MEDIA OF DIFFERING pH, OF *Mougeotia* 17μm.

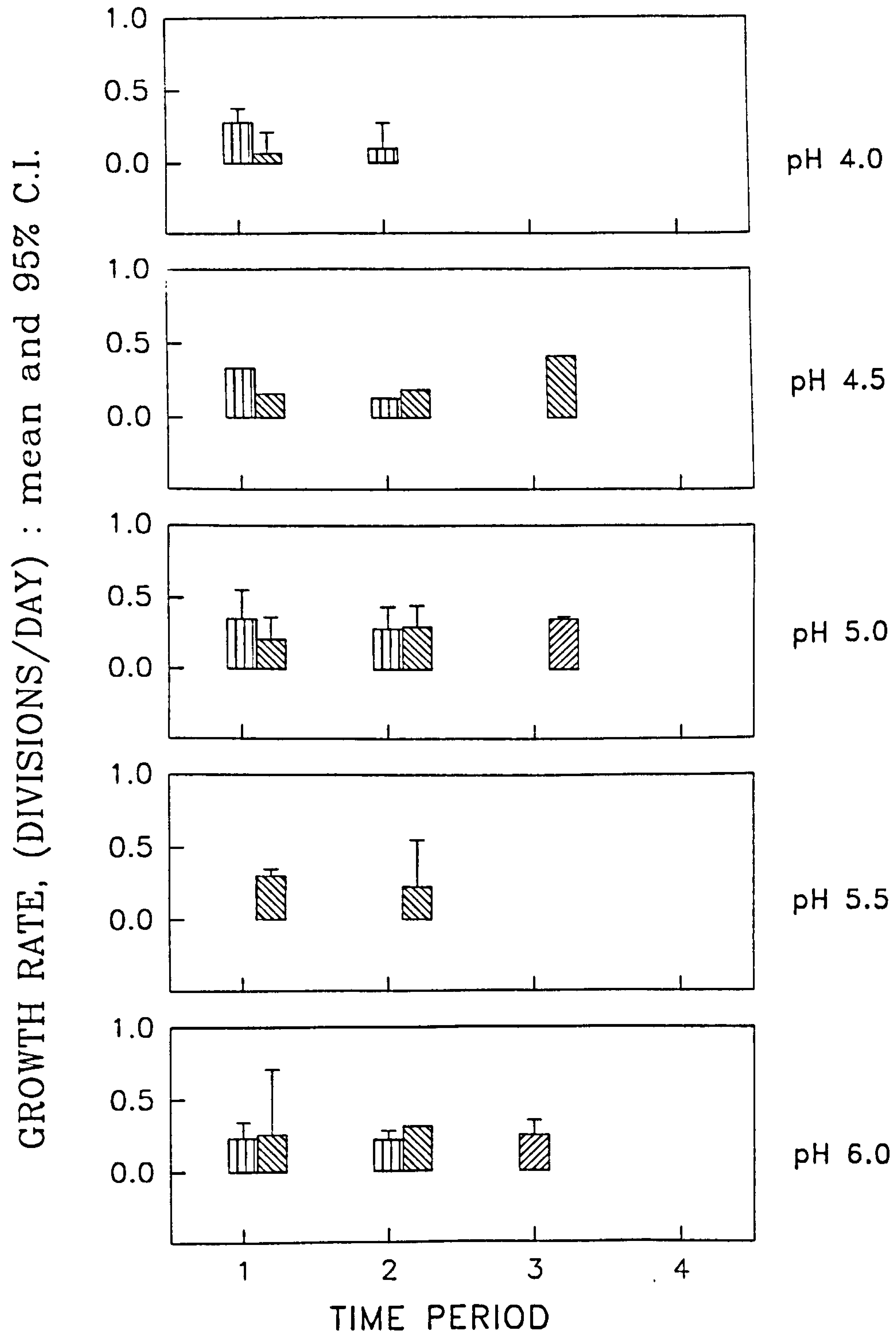


FIG.11:

GROWTH RATE DURING SEQUENTIAL PERIODS OF EXPOSURE TO MEDIA OF DIFFERING pH, OF *Mougeotia 22μm.*

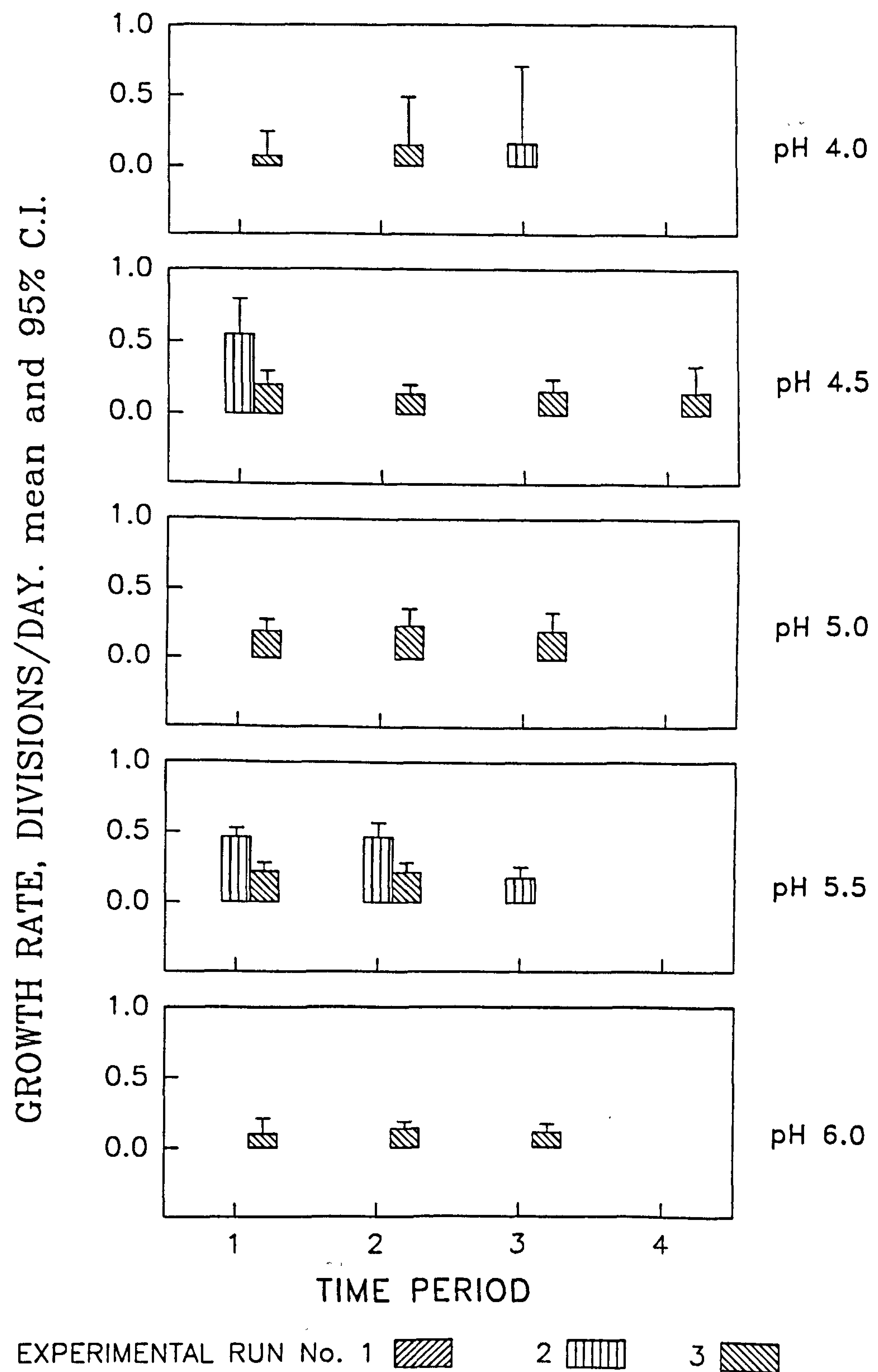


FIG.12 :
GROWTH RATE DURING SEQUENTIAL PERIODS OF EXPOSURE TO MEDIA OF DIFFERING pH, OF *Oedogonium* 6μm.

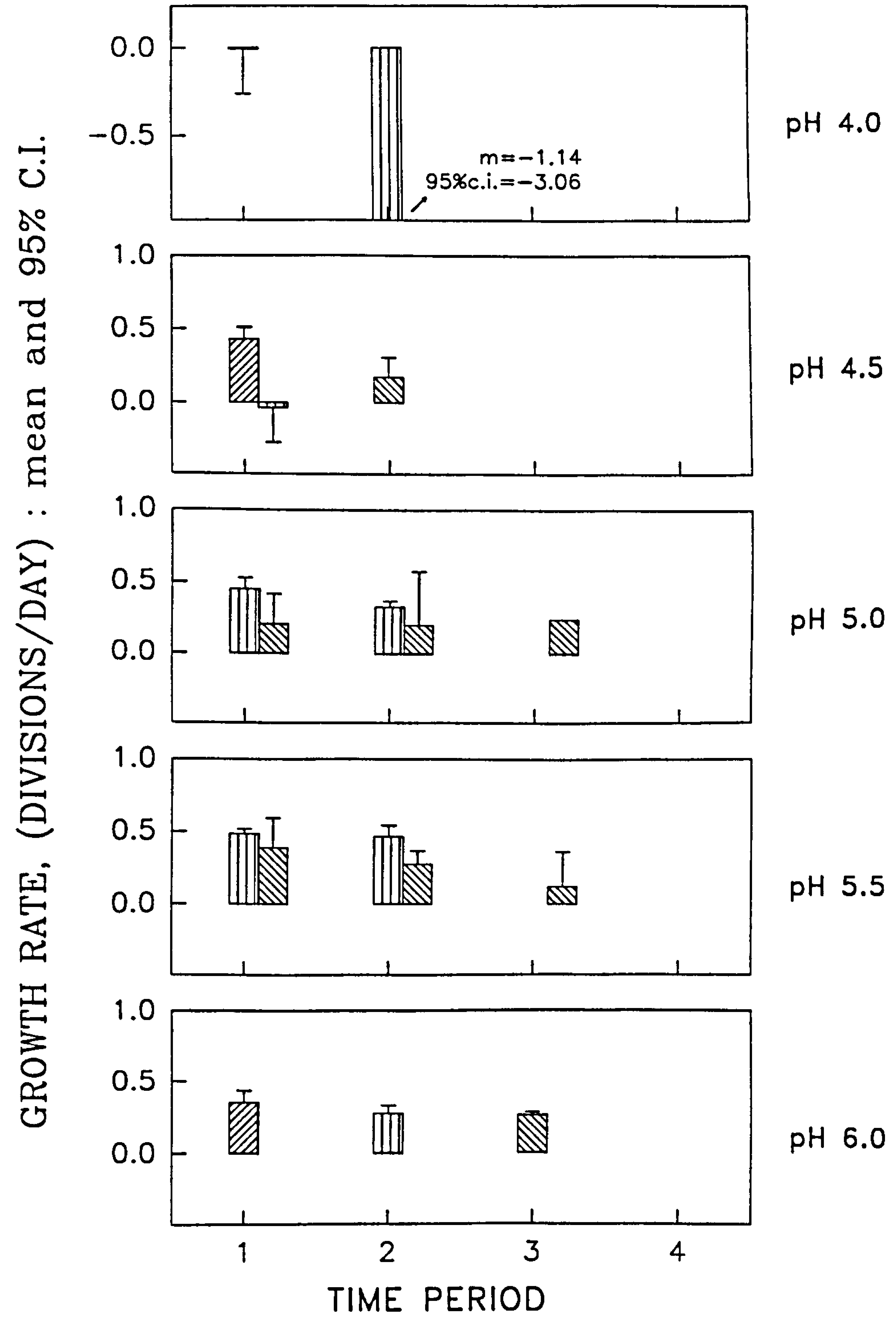


FIG. 13 :
GROWTH RATE DURING SEQUENTIAL PERIODS OF EXPOSURE TO MEDIA OF DIFFERING pH, OF *Oedogonium* 15μm.

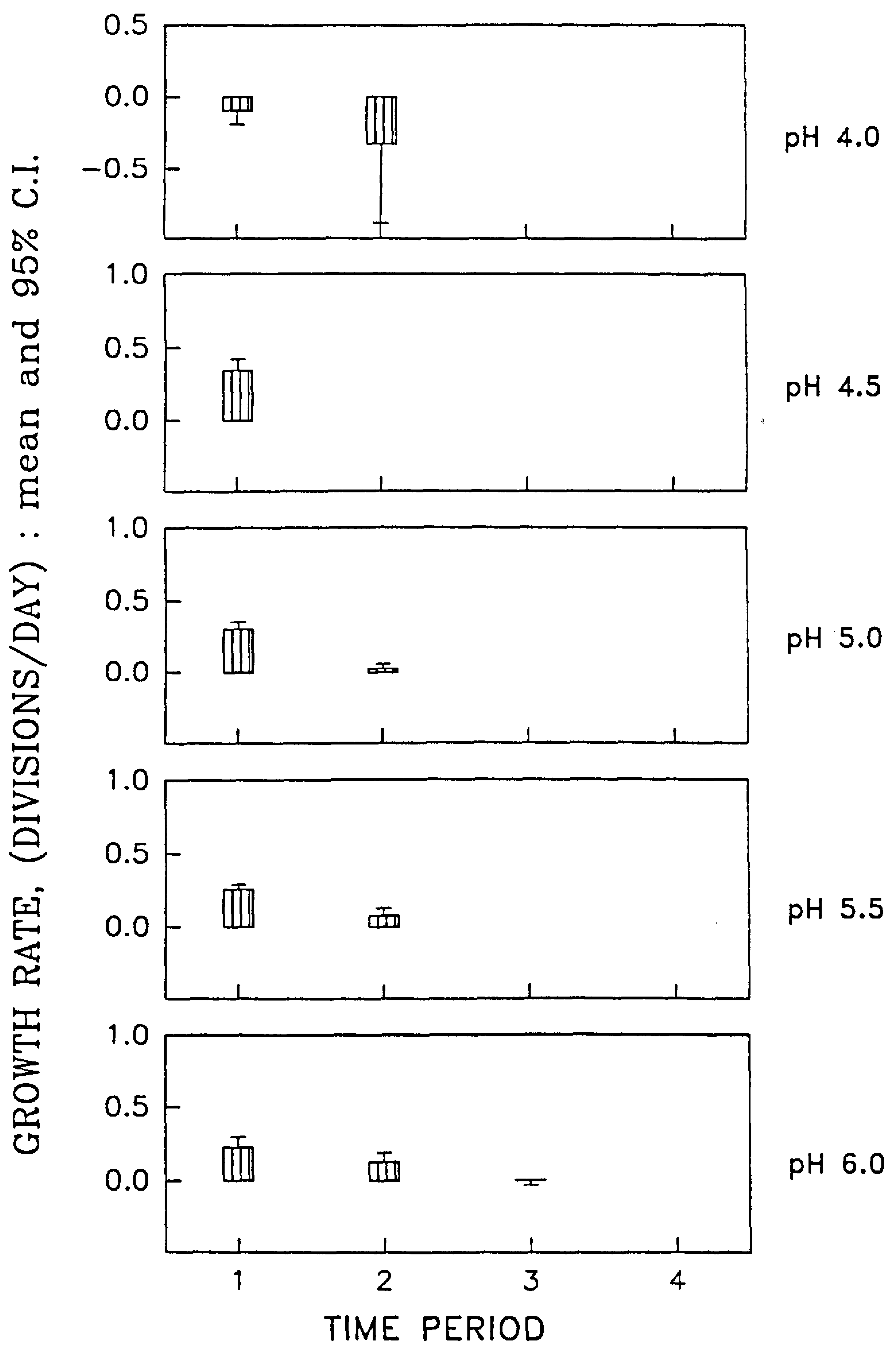


FIG.14:
GROWTH RATE OF FILAMENTOUS ALGAE
IN MEDIA OF DIFFERING pH.

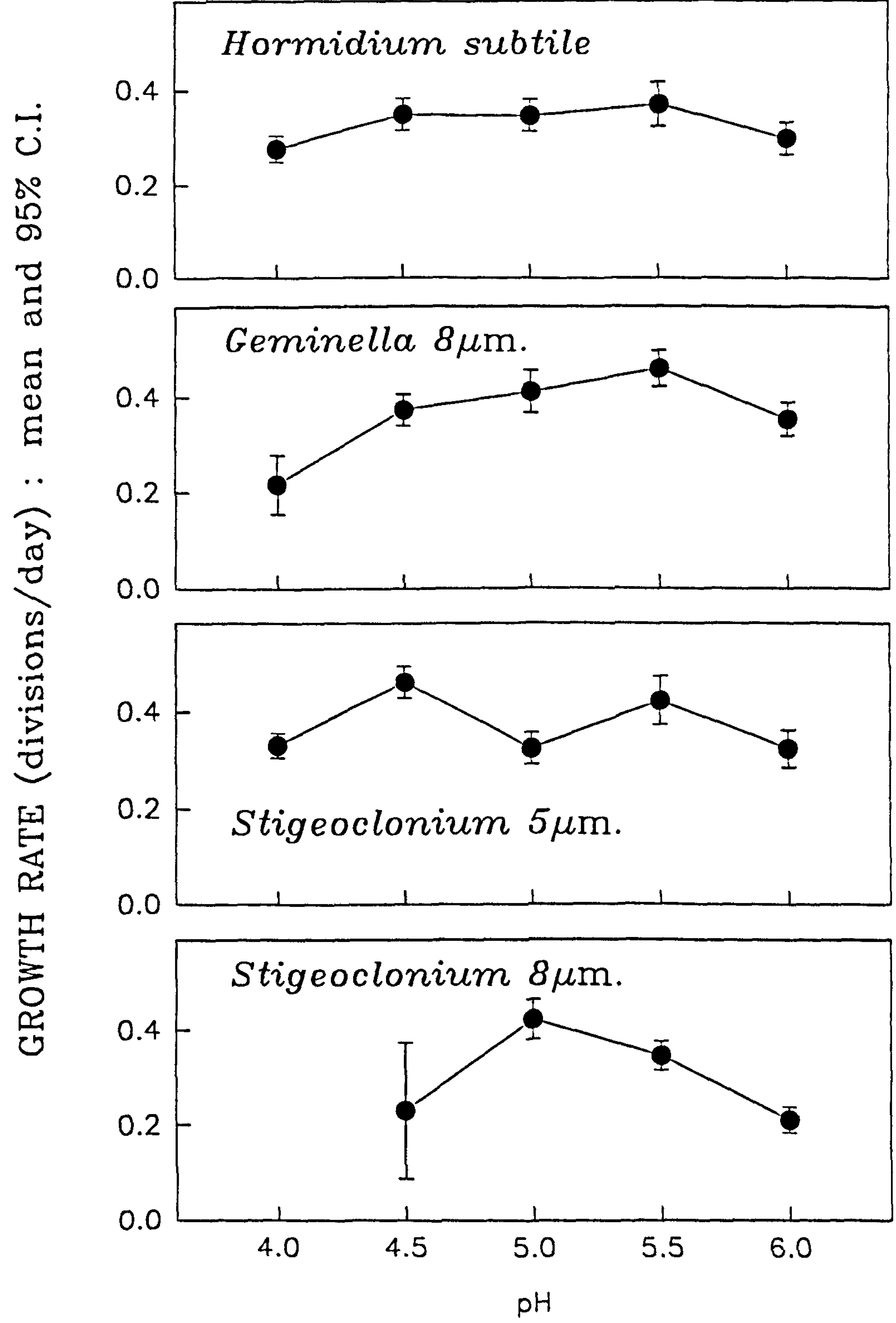
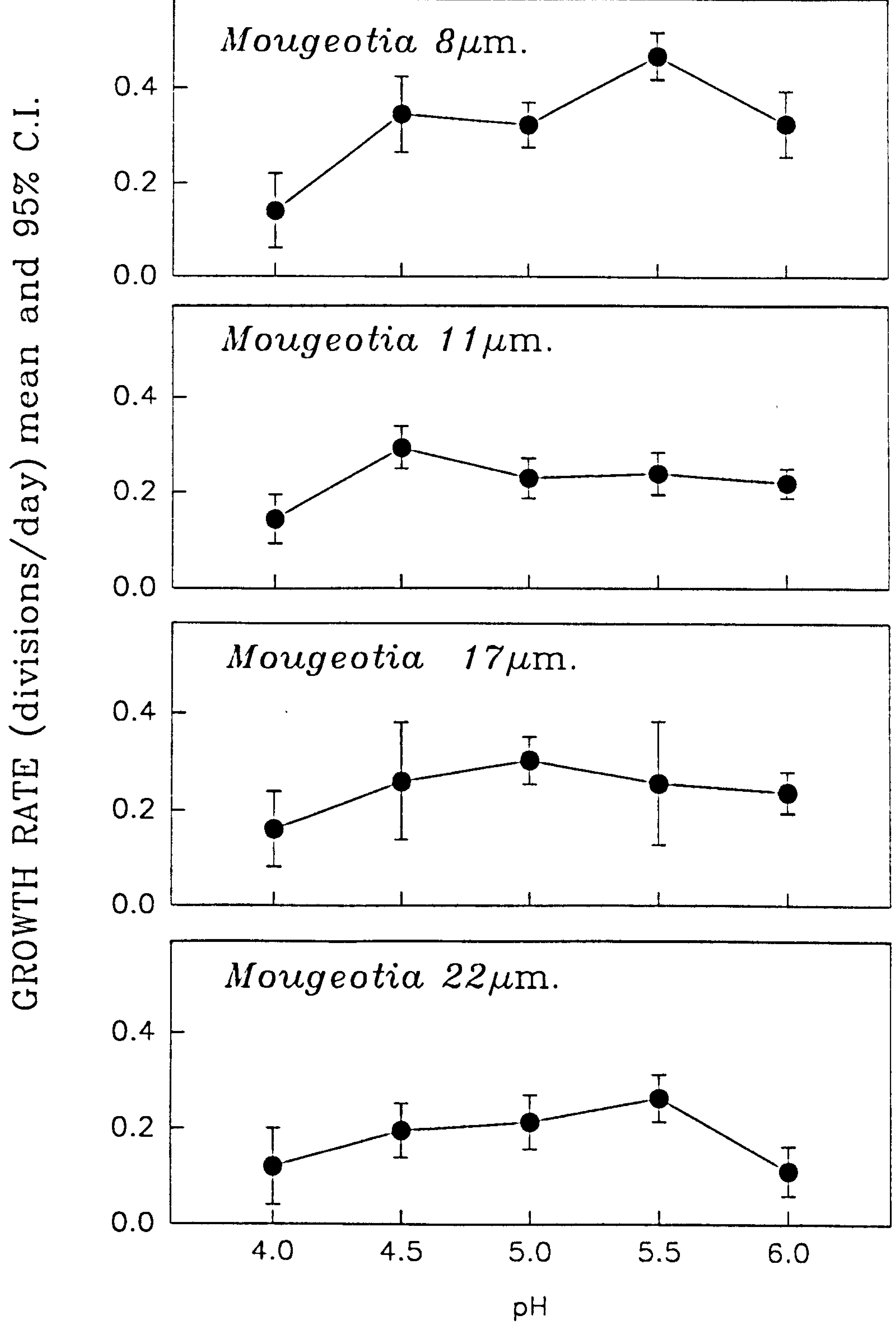


FIG.15

GROWTH RATE OF FILAMENTOUS ALGAE IN MEDIA OF DIFFERING pH.



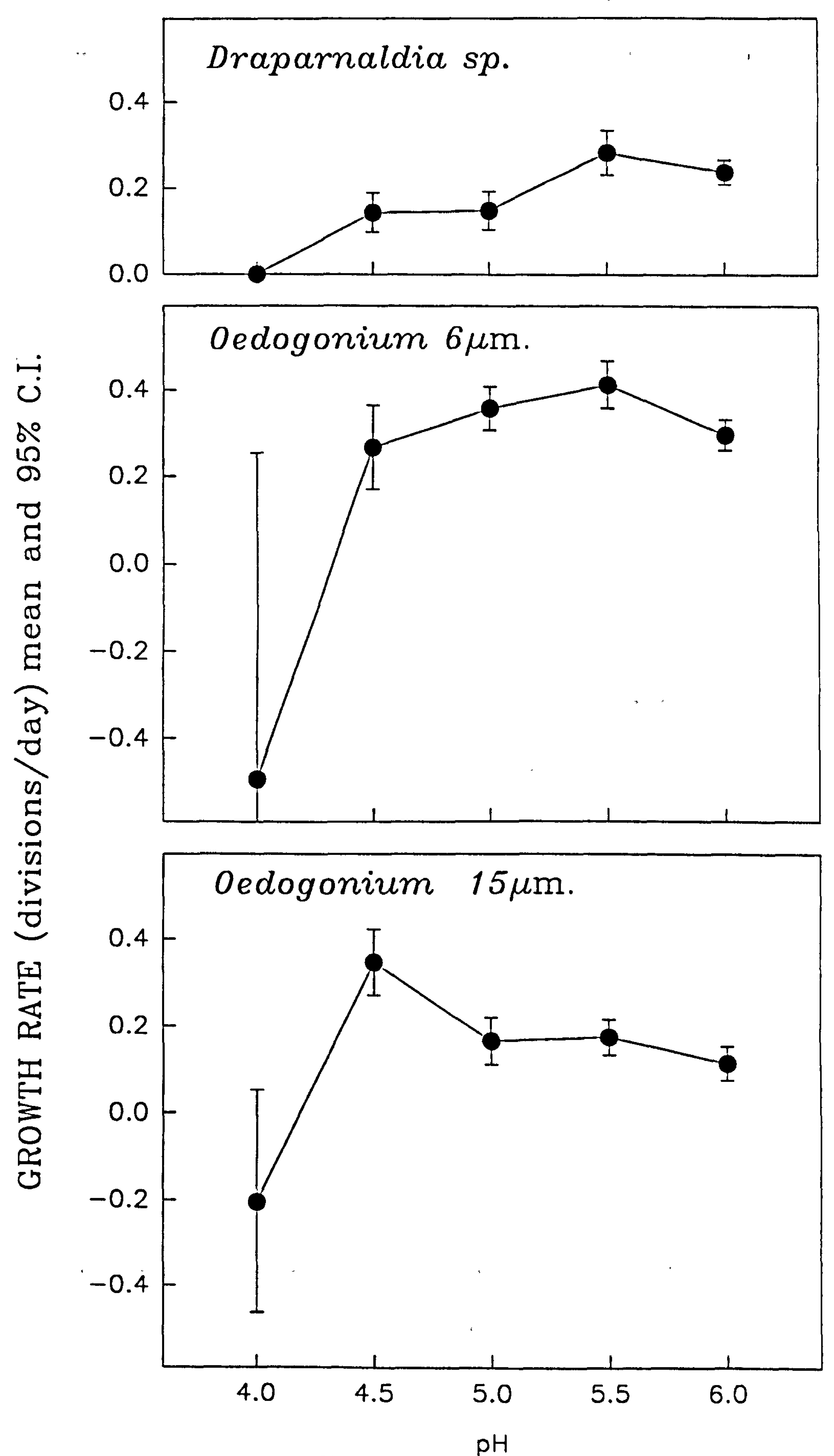


FIG.16
GROWTH RATE OF FILAMENTOUS ALGAE
IN MEDIA OF DIFFERING pH.
Draparnaldia sp.
Oedogonium 6μm.
Oedogonium 15μm.
GROWTH RATE (divisions/day) mean and 95% C.I.
0.4
0.2
0.0
−0.2
−0.4
4.0
4.5
5.0
5.5
6.0
pH

TABLE 21
GROWTH RATES ANALYSIS OF VARIANCE
pH MANIPULATION EXPERIMENTS

(a) MODEL ANOVA: GROWTH RATE = SPECIES * TREATMENT * TIME PERIOD + REPLICATE

Species: *Stigeoclonium* 5 µm; *Geminella* 5 µm; *Mougeotia* 11 µm; *Hormidium subtile*

	DF	S.SQ	M.SQ.	F	P
SPECIES	3	0.408	0.136	17.07	<0.001
TREATMENT	4	0.149	0.037	4.66	0.002
TIME PERIOD	1	0.075	0.075	9.36	0.003
INTERACTIONS:					
SP * TREATMENT	12	0.037	0.003	0.39	0.964
SP * TIME PERIOD	3	0.045	0.015	1.88	0.141
TREAT. * TIM. PER.	4	0.014	0.003	0.43	0.790
SP. * TREAT. * TIM.	12	0.015	0.001	0.16	0.999
REPLICATE	10	0.487	0.049	6.10	<0.001
ERROR	70	0.558	0.00		

Test normally distributed residuals: corr. (n. scores x resid.) = 0.984
n = 120
significant at 1% level

(b) MODEL ANOVA: GROWTH RATE = SPECIES * TREATMENT * TIME PERIOD + REPLICATE

Species: *Stigeoclonium* 5 µm; *Draparnaldia* sp; *Geminella* 8 µm; *Mougeotia* 5 µm; *Mougeotia*. 11 µm; *Hormidium subtile*

	DF	S.SQ	M.SQ.	F	P
SPECIES	5	0.537	0.107	25.76	<0.001
TREATMENT	4	0.448	0.112	26.84	<0.001
TIME PERIOD	1	0.009	0.009	2.07	0.156
INTERACTIONS:					
SP * TREATMENT	20	0.119	0.006	1.43	0.149
SP * TIME PERIOD	5	0.013	0.003	0.60	0.696
TREAT. * TIM. PER.	4	0.025	0.006	1.50	0.214
SP. * TREAT. * TIM.	20	0.032	0.002	0.38	0.990
REPLICATE	5	0.043	0.009	2.05	0.086
ERROR	55	0.229	0.004		

Test normally distributed residuals: corr. (n. scores x resid.) = 0.997
n = 120
significant at 10% level

TABLE 21 (cont.)
GROWTH RATES ANALYSIS OF VARIANCE
pH MANIPULATION EXPERIMENTS

(c) MODEL ANOVA: GROWTH RATE = SPECIES * TREATMENT + TIME PERIOD

Species: *Stigeoclonium* 5 µm; *Stigeoclonium.* 8 µm; *Draparnaldia* sp; *Geminella* 8 µm; *Mougeotia* 5 µm; *Mougeotia* 11 µm; *Mougeotia* 22 µm; *Hormidium subtile*

	DF	S.SQ	M.SQ.	F	P
SPECIES	7	0.514	0.073	58.41	<0.001
TREATMENT	4	0.267	0.067	33.16	<0.001
INTERACTIONS:					
SP * TREATMENT	28	0.110	0.004	3.12	0.001
TIME PERIOD		1	0.004	0.004	3.03 0.089
ERROR	39	0.049	0.001		

Test normally distributed residuals: corr. (n scores x resid.) = 0.999
n = 80
significant at 10% level

(d) MODEL ANOVA: GROWTH RATE = SPECIES * TREATMENT + TIME PERIOD

Species: *Stigeoclonium* 5 µm; *Draparnaldia* sp; *Geminella* 8 µm; *Mougeotia* 5 µm; *Mougeotia* 11 µm; *M.ougeotia* 22 µm; *Hormidium subtile*; *Oedogonium* 6 µm; *Oedogonium* 15 µm

	DF	S.SQ	M.SQ.	F	P
SPECIES	7	0.525	0.075	19.23	<0.001
TREATMENT	4	0.305	0.076	19.54	<0.001
INTERACTIONS:					
SP * TREATMENT	28	0.288	0.010	2.64	0.003
TIME PERIOD		1	0.032	0.032	8.18 0.007
ERROR	39	0.152	0.004		

Test normally distributed residuals: corr. (n scores x resid.) = 0.993
n = 80
significant at 10% level

TABLE 21 (cont.)
GROWTH RATES ANALYSIS OF VARIANCE
pH MANIPULATION EXPERIMENTS

(e) MODEL ANOVA: GROWTH RATE = SPECIES * TREATMENT + TIME PERIOD

Species: *Stigeoclonium* 5 μm; *Stigeoclonium* 8 μm; *Draparnaldia* ; *Geminella* 8 μm; *Mougeotia* 11 μm; *Hormidium subtile*

	DF	S.SQ	M.SQ.	F	P
SPECIES	5	0.456	0.091	13.99	<0.001
TREATMENT	4	0.086	0.022	3.31	0.016
INTERACTIONS:					
SP * TREATMENT	20	0.122	0.006	0.93	0.552
TIME PERIOD		2	0.151	0.075	11.56<0.001
ERROR	58	0.378	0.007		

Test normally distributed residuals: corr. (n scores x resid.) = 0.987
n = 90
significant at 10% level

(f) MODEL ANOVA: GROWTH RATE = SPECIES x TREATMENT + TIME PERIOD

Species: *Stigeoclonium* 5 μm; *Geminella* 8 μm; *Mougeotia* 5 μm; *Hormidium subtile*

	DF	S.SQ	M.SQ.	F	P
SPECIES	3	0.523	0.174	25.85	<0.001
TREATMENT	4	0.036	0.009	1.34	0.265
INTERACTIONS:					
SP * TREATMENT	12	0.020	0.002	0.25	0.994
TIME PERIOD		3	0.310	0.103	15.29<0.001
ERROR	57	0.385	0.007		

Test normally distributed residuals: corr. (n scores x resid.) = 0.978
n = 80
significant at 1% level

TABLE 22
CHANNEL GROWTH EXPERIMENTS; CHEMISTRY
pH EXPERIMENTS

CHANNEL pH	1 4.6 (B9)		2 4.0		3 4.5		4 5.0		5 5.5		6 6.0	
INITIAL/FINAL	i	f	i	f	i	f	i	f	i	f	i	f
RUN 1												
TON $\mu eq\ l^{-1}$	1.5		18.6		20.0		19.3		17.3		20.0	
PO_4 "	7.7		0.9		1.0		1.2		0.9		0.8	
RUN 2												
TON $\mu eq\ l^{-1}$	-3.6	3.0	13.6	13.4	27.9	26.1	26.6	26.4	26.4	29.8	31.5	29.4
PO_4 "	7.7	7.6	1.6	1.4	1.3	1.1	1.4	1.3	1.6	1.5	1.6	1.5
RUN 3												
TON $\mu eq\ l^{-1}$	ND	5.7	25.1	23.5	21.5	19.3	16.9	14.9	19.9	19.4	19.6	18.4
PO_4 "	6.6	11.8	0.7	0.6	0.9	0.9	0.5	0.5	0.7	0.8	0.6	0.6
Al-TM $\mu g.\ l^{-1}$	55.6	51.6	14.2	23.2	ND	4.6	1.6	3.0	0.6	3.0	0.5	ND
$\mu eq l^{-1}$	6.18	5.73	1.58	2.58		0.51	0.18	0.33	0.07	0.33	0.06	
AL-NL $\mu g.l^{-1}$	1.5	0.3	0.5	-		*	*	*	*	*	*	
$\mu eq l^{-1}$	0.17	0.03	0.06	-		*	*	*	*	*	*	
Al-L $\mu g.l^{-1}$	54	51.3	13.7	23.2		4.6	1.6	3.0	0.6	3.0	0.5	
$\mu eq l^{-1}$	6.00	5.70	1.52	2.58		0.51	0.18	0.33	0.07	0.33	0.06	

*Fractionation not carried out because of low [Al-TM]

B9 = Treatment D in Al experiments   ND = Not detectable

3.4.2 Effect of Al on Growth Rate

Three treatments based on Corrie mediuim were used, all at pH 5.5. Treatment A was unmodified, B had the silicate concentration reduced to approximately one-tenth, and C had, in addition, total aluminium input increased to equal that of medium B9. The concentrations measured were found to differ from the target values (table 24). In addition, the results of measurements made in parallel with the pH experiments, in B9, are shown alongside, as treatment D. The Al experiments (Figs. 17-22 and Table 23) demonstrate the profound influence which total [Al] of approximately 200 µg l^{-1} exerts on the growth rate of all species. Only *Hormidium subtile* and*Geminella 8* µm. were able to grow under these conditions(treatment C), at a reduced growth rate. In contrast, in the B9 medium (treatment D), other species including *Mougeotia* 5µm, *Mougeotia* 11µm and *Stigeoclonium* sp. grew readily.

Burn 9, the prototype for B9 medium, has a high mean PO_4 concentration, possibly due in part to fertilization of the catchment by the Forestry Commission (pers. comm.). Nutrient leaching may continue for several years after such a treatment (Harriman, 1978).

The composition of the media with respect to important chemical parameters at the beginning and end of the growth measurements is shown in Table 24. Particularly striking is the difference in growth rate between the two *Stigeoclonium* isolates, 9e and 10a, in treatment C. *Stigeoclonium* 9e originated in Burn 9, an acidified but humic stream, also with high PO_4 levels, both of which may ameliorate the effects of aluminium, while *Stigeoclonium* 10a was isolated from Burn 10, which has the highest mean Al concentration, particularly as Al-L, of all sites (Tables 10-12).

During the earlier series of experiments on the effect of pH, the strain of *Stigeoclonium* was not well defined, samples from Burn 14, Burn 9 and Burn 11 being mixed in some inocula. Therefore it is not certain which strain is represented in the graph of growth in medium B9 (treatment D). The lower growth rate seen in B9 (0.3 to 0.6 d/d) in comparison with treatments A and B must be compared with that obtained in the pH experiments at pH 4.5, i.e. approximately 0.45 d/d. One-way analysis of variance carried out on the pH experiments shows that the growth rate was not significantly different between pH 4.0, pH 4.5 (Corrie medium) and B9 at pH 4.6, so that the lower growth rate in D (Fig. 20) may be due to the pH difference between C and D. It may be that the different growth rates seen in different experimental runs on the effects of pH, on most species, may result from different sources of the algae used in the inocula.

The existence of ecotypes has been well documented, e.g. for *Stigeoclonium*, and may apply to some extent also for other species or genera. This means that exact conclusions cannot be drawn from these experiments about the extent of influence which pH and aluminium exert on the growth rate of a particular species. It would also blur the indicator values of species, for example in analyses by CANOCO.

FIG. 17 : GROWTH RATE RESPONSE OF *Hormidium subtile* IN MEDIA CONTAINING DIFFERING LEVELS OF Si AND Al.

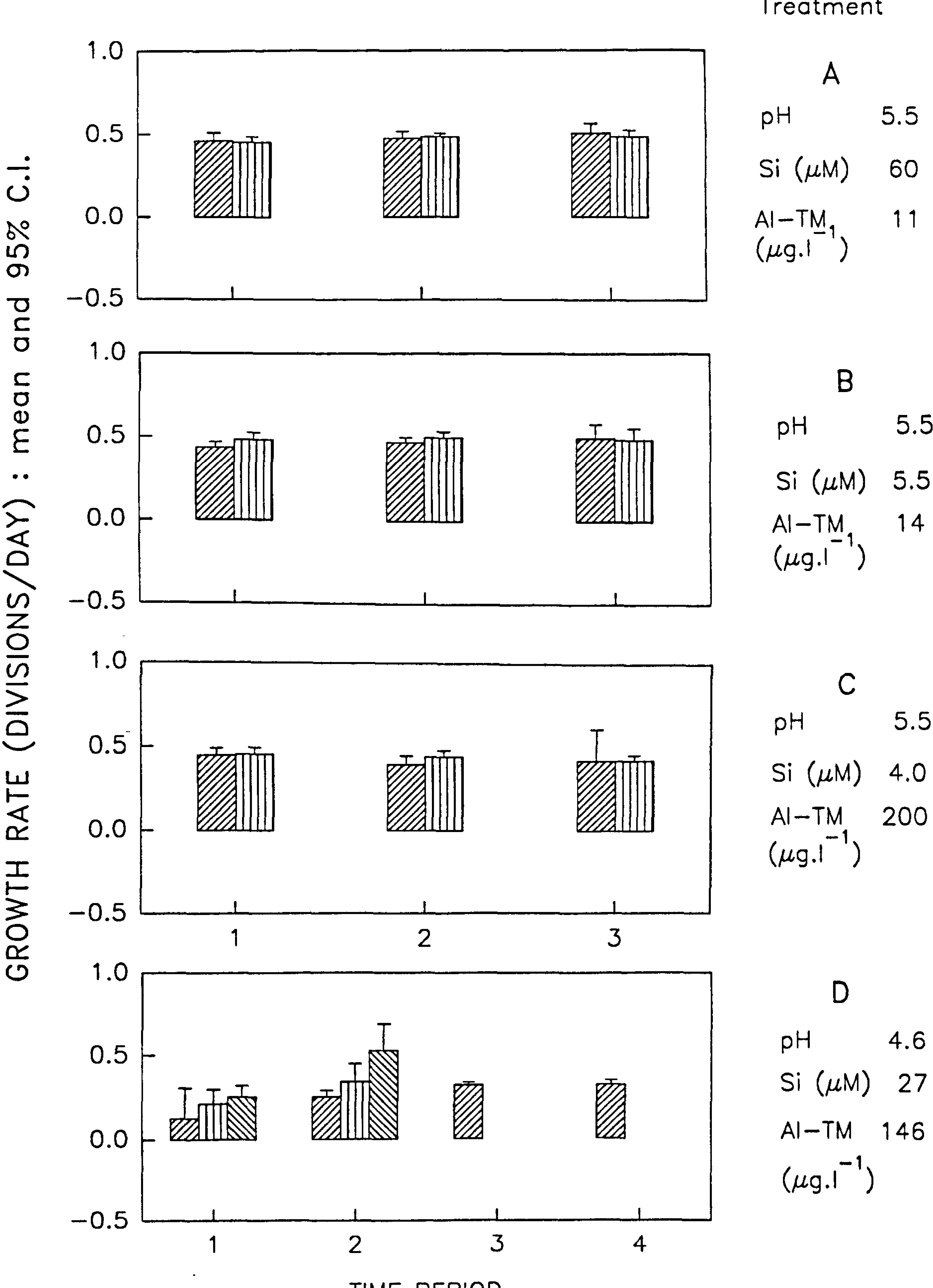


FIG. 18 : GROWTH RATE RESPONSE OF *Geminella* 8μm. IN MEDIA CONTAINING DIFFERING LEVELS OF Si AND Al.

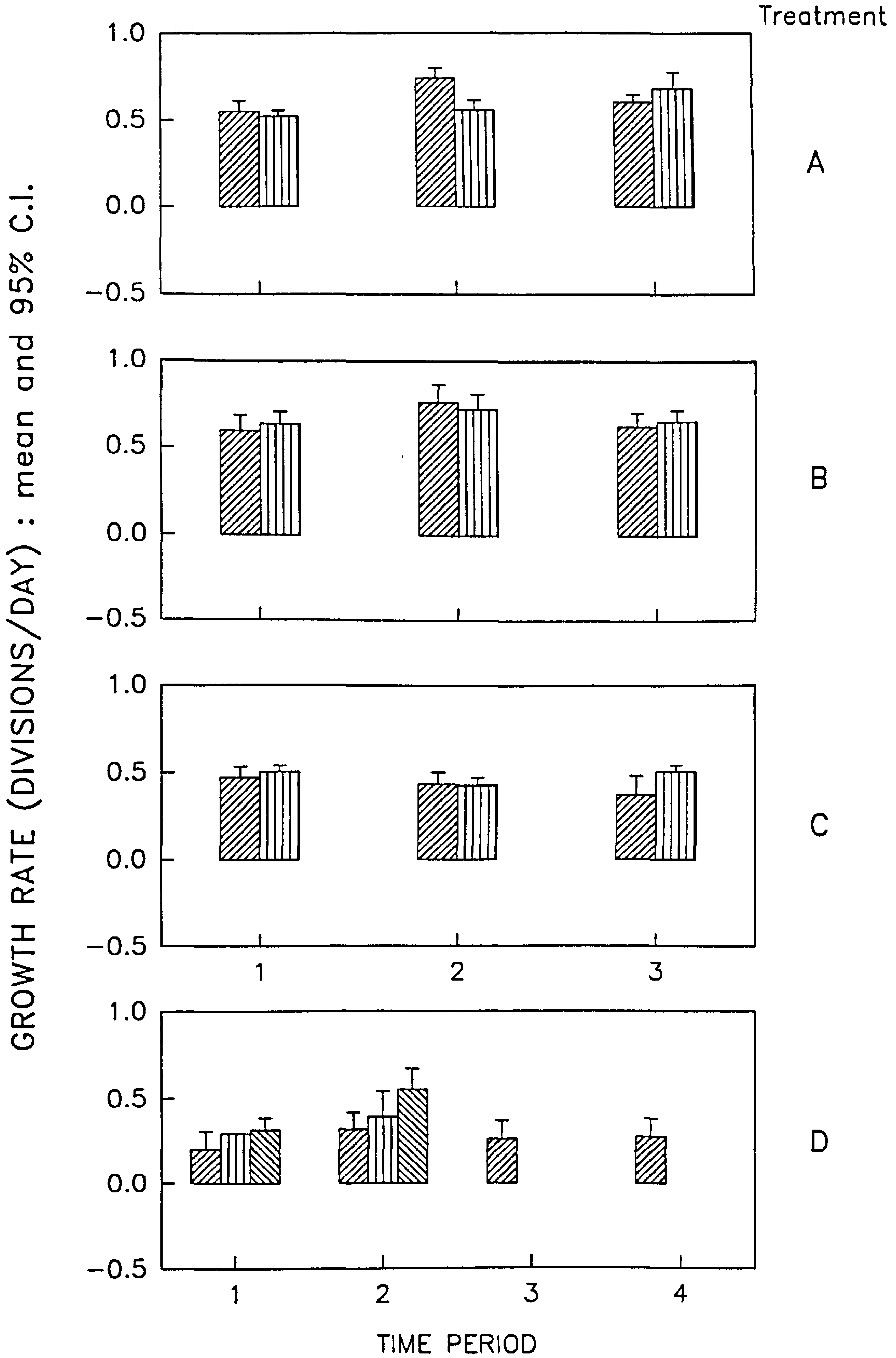


FIG.19

GROWTH RATE RESPONSE OF *Stigeoclonium 9e* IN MEDIA CONTAINING DIFFERENT LEVELS OF Si AND Al.

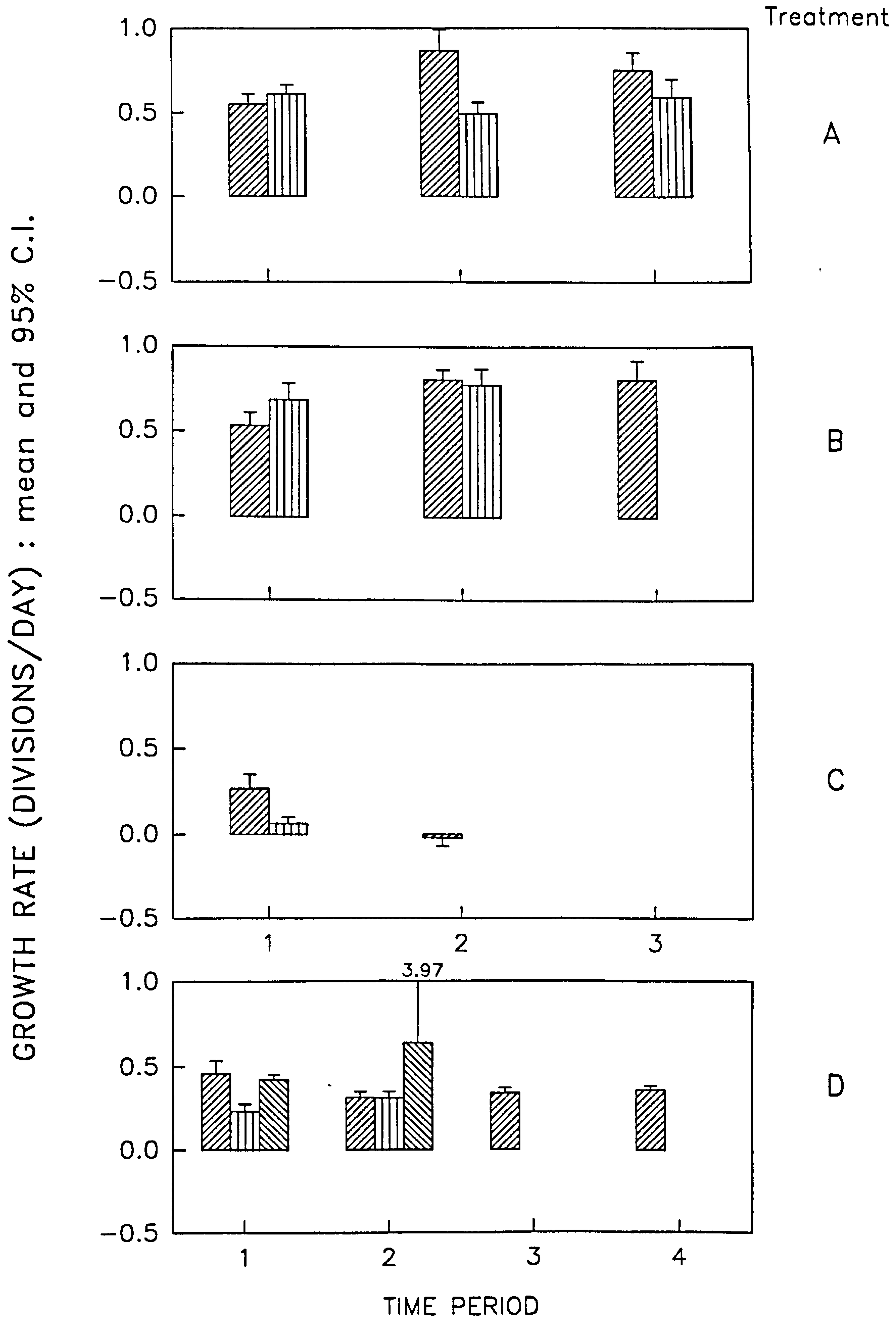
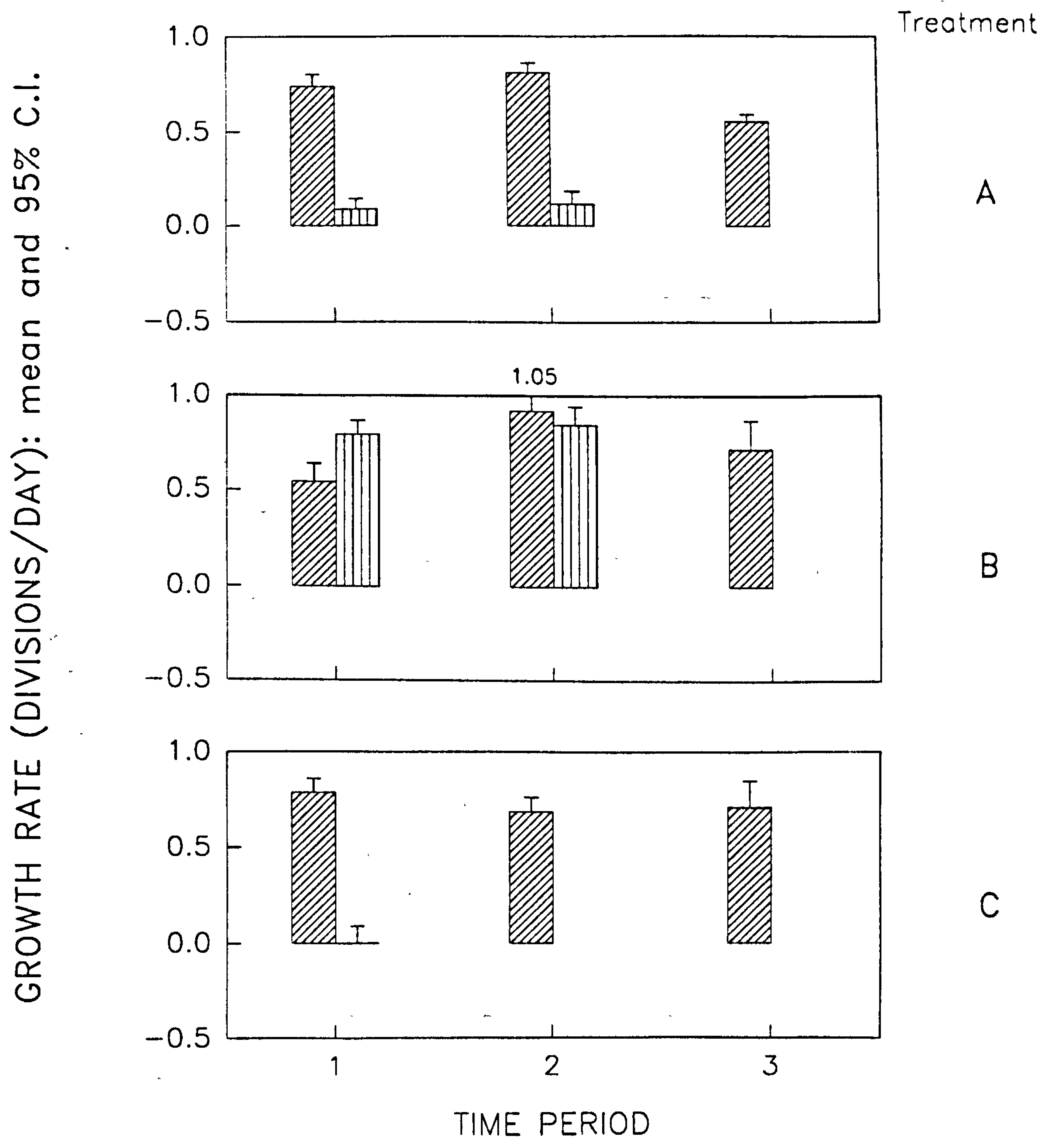


FIG.20
GROWTH RATE RESPONSE OF *Stigeoclonium 10a* IN MEDIA CONTAINING DIFFERENT LEVELS OF Si AND Al.



(TREATMENT D, GROWTH IN MEDIUM B9: SEE GRAPH OF *Stigeoclonium 9e*. NO DIFFERENTIATION BETWEEN ISOLATES FROM BURNS 9 AND 10 WAS MADE IN THE EARLIER PART OF THE WORK, WHEN THE GROWTH RATES IN B9 WERE MEASURED)

FIG. 21: GROWTH RATE RESPONSE OF *Draparnaldia sp.* IN MEDIA CONTAINING DIFFERENT LEVELS OF Al AND Si.

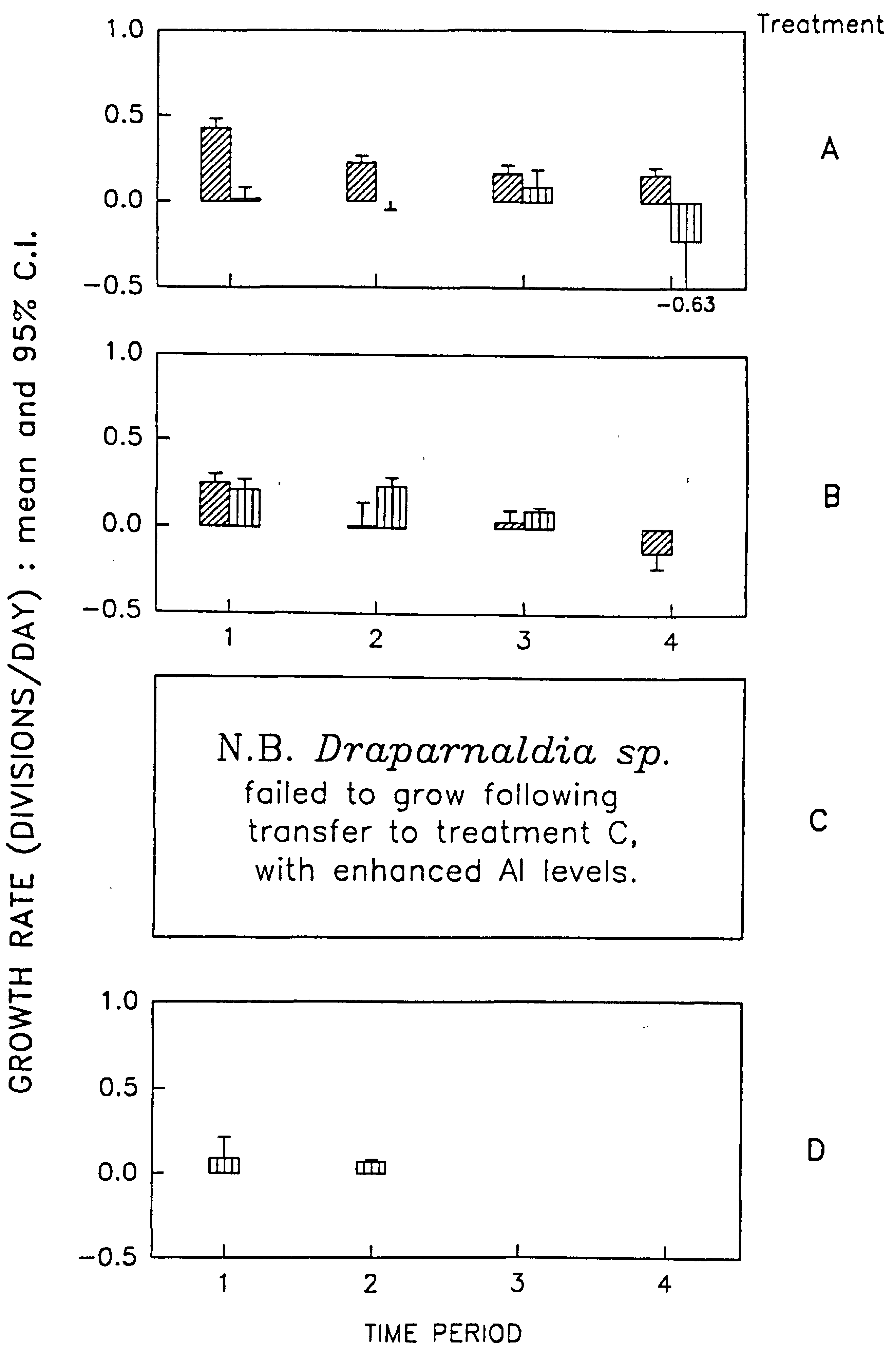


FIG. 22 :
GROWTH RATE RESPONSE OF *Mougeotia* 8μ IN MEDIA CONTAINING DIFFERING LEVELS OF Si AND Al.

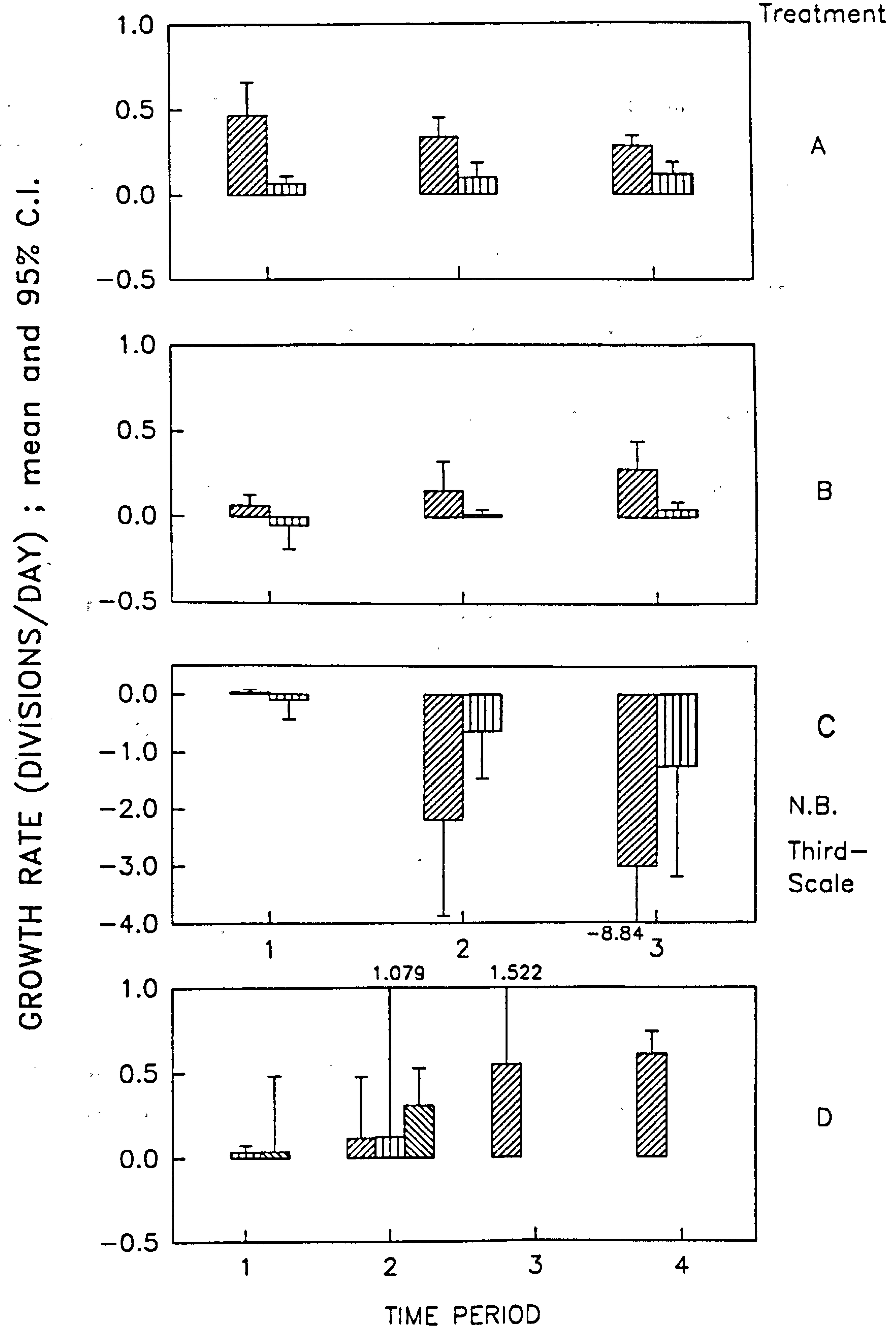


TABLE 23
ANALYSIS OF VARIANCE ON GROWTH
RATES FROM Al/Si MANIPULATION

MODEL ANOVA: GROWTH RATE = SPECIES * TREATMENT * TIME PERIOD + REPLICATE

Species: *Stigeoclonium* (9e) and (10a); *Draparnaldia* sp.; *Geminella* 8μm.; *Hormidium subtile*; *Mougeotia* 5μm.;

	D.F.	S.SQ.	M.SQ.	F	P
SPECIES	7	5.132	0.733	27.01	<.001
TREATMENT	2	2.134	1.067	39.31	<.001
TIME PERIOD	2	0.435	0.217	8.01	.001
INTERACTIONS:					
SP * TREATMENT	14	2.949	0.211	7.76	<.001
SP * TIME PERIOD	14	0.770	0.055	2.03	0.028
TREAT. * TIM. PER.	4	0.208	0.052	1.92	0.117
SPEC. * TREAT. * TIM.	28	1.724	0.062	2.27	0.003
REPLICATE	1	.000	0.000	0.00	0.980

Test normally distributed residuals: corr (n scores x residuals) = 0.854
n = 144
not significant at 1% level*

* negative growth rates were included in this analysis.
Conversion of negatives to zeroes greatly improves normality (corr. = 0.926)

TABLE 24
CHANNEL GROWTH EXPERIMENTS; CHEMISTRY
Al/Si MANIPULATION EXPERIMENT

	MEASURED VALUES												TARGET VALUES			
CHANNEL	1		2		3		4		5		6					
TREATMENT	A		B		C		A		B		C		A	B	C	D
INITIAL/FINAL	i	f	i	f	i	f	i	f	i	f	i	f	/	/	/	/
TON µeq l^{-1}	27.6	26.4	24.8	24.0	26.8	26.8	27.0	26.8	26.0	24.6	29.0	26.2	30	30	30	8
PO_4 "	1.6	1.8	0.9	0.5	0.7	<0.2	1.2	0.7	1.0	0.7	0.4	0.3	5	5	5	45
Al-TM µg l^{-1}	8	14	11	10	190	226	11	12	11.5	19	172	226	40	40	146	146
µeql^{-1}	0.89	1.56	1.22	1.11	21.11	25.11	1.22	1.33	1.28	2.11	19.11	25.11	4.44	4.44	16.2	16.2
Al-NL µg l^{-1}	*	*	*	*	8	11	*	*	*	*	13	14	/	/	/	/
µeql^{-1}	*	*	*	*	0.89	1.22	*	*	*	*	1.44	1.56				
Al-L µg l^{-1}	*	*	*	*	182	215	*	*	*	*	159	212	/	/	/	/
µeql^{-1}	*	*	*	*	20.22	23.89	*	*	*	*	17.67	23.56				
SiO_2 µM	59.0	62.5	5.5	5.5	4.0	4.0	57.0	60.0	5.0	5.5	4.0	4.0	47	4	4	27

*Fractionation not carried out

Nevertheless the general conclusions are clear: (a) species differ in their ability to grow at pH values between 4.0 and 6.0; (b) they differ also in their ability to grow in the presence of concentrations of Al which may be found in some acidified streams; and (c) these differences in growth rates are related to the conditions pertaining in the streams in which the species are most abundant.

3.4.3 Species composition on continued culture

The issue of whether the growth rates measured during the early phases of growth are maintained as the biomass develops cannot easily be determined for technical teasons. However a subjective estimate of relative species composition has been carried out on channel biomass in the same way as with field samples for Run 2, which was continued for 29 days (Table 25), and for Run 3, continued for 64 days (Table 26). These populations are not naturally balanced as they were deliberately seeded with particular species; any others present were there as contaminants.

Considering the results of both experiments, at pH values below 5.0 *Stigeoclonium* 5µm was dominant, together with *Hormidium* 5.5µm. At pH values above 5.0, *Mougeotia* 11µm becomes codominant with *Stigeoclonium.*, while *Hormidium* declines slightly in abundance.

Mougeotia 8µm, *Mougeotia* 17µm and *Mougeotia* 22µm are absent from pH 4.0. *Mougeotia* 22µm has a very patchy distribution and low abundance, surprising in view of its considerable contribution to the community in medium B9 in Run 3.

In run 3, two *Stigeoclonium* taxa, distinguished here by their main axis diameters, occur only at higher pH values. These may be separate ecotypes, adapted to a higher pH, or they may be morphological responses to the higher pH. In the early stages of its growth, *Draparnaldia* cannot be readily distinguished from *Stigeoclonium*, and indeed fails to develop main axis differentiation if the calcium concentration is below 1.7 mg l^{-1} (Johnstone, 1978). Therefore the largest *Stigeoclonium* taxon may in fact be *Draparnaldia*.. *Zygnema* 20 µm is present at pH 4.0 and common at pH 4.5 but not at higher pH..

Pseudanabaena in these experiments is seen to occur at all pH values, including pH 4.0, and is at a maximum at pH 5.0 although it is absent from B9 in Run 3. This cyanophyte was largely ignored in field samples during the earlier phase of the sampling programme, but has repeatedly cropped up in cultures. In field samples it is quite difficult to score due to its small cell diameter (1.5 µm) and may be readily confused with other small cyanophytes. Many of the records of '*Oscillatoria* 1µm' probably refer to *Pseudanabaena* especially in Matrix B.

TABLE 25
CHANNEL GROWTH EXPERIMENT: FINAL SPECIES COMPOSITION
RUN 2

	B9	CORRIE : pH				
SPECIES		4.0	4.5	5.0	5.5	6.0
Hormidium 5.5 µ0m.	+++	++++	++++	+++	++	++
Geminella 8 µm.	+	++	++	++++	+	+++
Geminella 11 µm.	-	-	+	+	-	-
Microspora 8 µm.	+	++	++	+	+	+
Mougeotia 8 µm.	-	-	-	+	++	-
M. 11 µm.	++	+	+	++++	+++	+++
M. 17 µm.	+	-	-	+	+	+
M. 22 µm.	-	-	-	+	+	-
Stigeoclonium 5 µm.	+++	++++	++++	++	+++	+++
Draparnaldia	+	-	+	+	++	+
Spirogyra 25 µm.	-	-	+	-	-	-
Oedogonium 6µm.	-	-	-	-	+	+
Oscillatoria 3 µm.	-	-	-	-	-	+
O. 8 µm.	-	-	-	-	+	+
Pseudanabaena 1.5 µm.	+	++	++	+	+	+

TABLE 26

CHANNEL GROWTH EXPERIMENT: FINAL SPECIES COMPOSITION, RUN 3

	B9	CORRIE : pH				
SPECIES		4.0	4.5	5.0	5.5	6.0
Hormidium 5.5 µm.	++++	+++	+++	+++	+++	+++
Geminella 8 µm.	-	++	+++	++	+++	++
Microspora 8 µm.	++	-	++	+	++	+
Mougeotia 8 µm.	+	-	++	+++	+++	+++
M. 17 µm.	-	-	-	+++	-	++
M. 22 µm.	+++	+	-	+	-	-
Stigeoclonium 5 µm.	++++	++++	++++	++++	++++	++++
S. 8-11 µm.	-	-	-	++	++++	-
S. 14 µm.	-	-	-	-	-	+++
Zygnema 20 µm.	+	-	+	+++	-	-
Pseudanabaena 1.5 µm.	-	++	++	+++	++	++

3.4.4 Influence of Channel pH on Biomass

Biomass developed at different pH in channels after continued incubation is shown in Fig. 23, expressed as mean daily increment in order to enable comparisons to be made between Runs 2 and 3, with the total biomass figures shown in Table 27.

Chlorophyll was at a maximum at pH 5.0 in Run 2 and pH 4.5 in Run 3. Carotenoid was at a maximum at pH 5.5 in Run 2, but in Run 3 there was very little change with pH, except that the lowest Chl.a value is found at pH 4.0

AFDW in Run 2 follows closely carotenoid, with a maximum at pH 5.5. In Run 3 AFDW rises sharply from a low at pH 4.0 to a maximum at pH 5, with a slight drop thereafter.

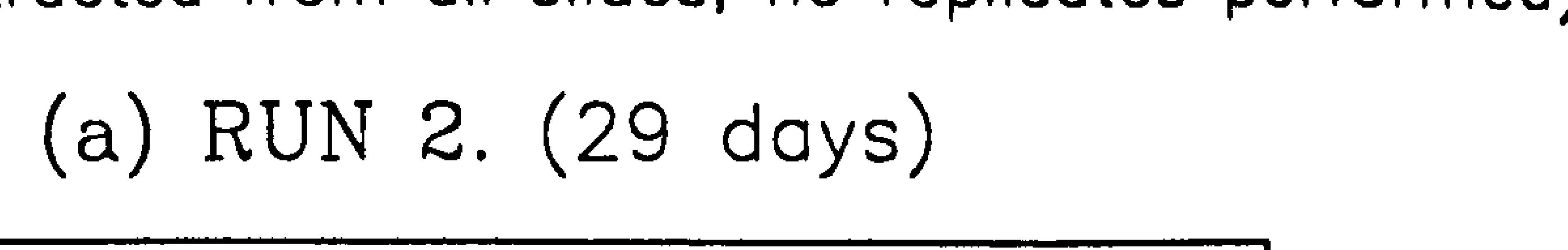
In both runs, the chlorophyll maximum occurs at a lower pH (0.5 unit) than the carotenoid/AFDW maximum. This may reflect a different species composition at the different pHs, as green algae have a higher ratio of chlorophyll to dry weight than cyanophytes or diatoms. This result is also found in the tub growth experiments.

The results do not support the contention that biomass is greater at low pH. Rather, within the limits of the species composition of periphyton developed during these experiments, it shows that biomass is at a maximum around the middle of the pH range studied here. The maximum growth rate of many of these species also occurs around this pH, and it thus seems likely that it is pH preference which determines the accumulation of biomass. In these channels no grazing organisms were present, with the exception of some rotifers. Therefore if decreased invertebrate grazing were the cause of 'blooms' of green algae present in natural streams, no difference should have been seen between the amounts of chlorophyll developed at different pH in the channels. On the other hand, if reduced heterotrophic activity at low pH were the cause of (supposed) biomass accumulation (Hendrey, 1976), the biomass ought to be at a maximum at pH 4, while this is clearly seen not to be the case.

The evidence from these experiments as well as from the field work, suggests that there is no consistent change in field biomass with pH, and that changes in species composition are responsible for the apparent increase in algal abundance in acidified streams. While effects of pH on grazing and on microbial decomposition may occur, the major cause of species shifts is the differential effect of pH, aluminium and/or other environmental factors on the growth rate of algae.

FIG.23 : BIOMASS DEVELOPED ON SLIDES IN CHANNELS, MEAN DAILY INCREMENT.

(Total biomass extracted from all slides, no replicates performed)



(a) RUN 2. (29 days)

CHLOROPHYLL a, μg. : CAROTENOID, μspu.

ASH–FREE DRY WEIGHT, mg.

0 2 4 6 8

0.0 0.5 1.0

4.0 4.5 5.0 5.5 6.0

pH

(b) RUN 3. (64 days)

0 2 4 6 8

0.0 0.5 1.0

4.0 4.5 5.0 5.5 6.0

pH

Chl.a : ○ in Corrie medium: ● in B9

Carot. : △ " " " ▲ " "

AFDW : □ " " " ■ " "

TABLE 27
FINAL CHANNEL BIOMASS
DEVELOPED IN pH EXPERIMENTS

	RUN 2 = 29 days			RUN 3 = 64 days		
MEDIUM/ pH	Chl.a µg.	Carot. µspu.	AFDW mg.	Chl.a µg.	Carot. µspu.	AFDW mg.
B9 4.6	39.4	12.0	1.5	73.0	32.2	26.7
Corrie 4.0	93.3	29.0	2.7	343.4	108.9	17.3
4.5	125.4	64.9	9.1	574.0	194.4	48.5
5.0	216.0	73.1	8.0	392.7	177.6	78.3
5.5	108.5	107.2	18.4	363.4	149.8	49.5
6.0	63.3	22.2	1.9	322.5	152.5	65.0

Figures shown are values for total biomass present on all slides in each channel.

3.5 TUB GROWTH EXPERIMENTS

Preliminary experiments on the methodology of growth rate measurement were carried out in tub growth experiments, with 2 l of medium mixed by airlift pump (see Methods, Section 2.6.2). The primary reason for carrying out the tub growth experiments however was to invesigate the effects of pH on biomass and relative species abundance. Two experiments were run, the first using medium B9 and pH-adjusted Corrie, the second involving the additional variable of the presence of Germanium, added to 5µg.l^{-1} as GeO_2 dissolved in 0.02N NaOH.

Final biomass developed at different pH in tub experiment 1 is shown in Fig. 24a and Table 30a. Carotenoid and AFDW show an increase with increasing pH. Chlorophyll is greatest at pH 4.4-4.8. The biomass values have been expressed as biomass units per litre of medium used, since batch replacement of the medium was practised over an extended period (20 l. over 18 weeks).

The final species composition is shown in Table 28. At low pH, 4.0 to 4.4, *Hormidium* dominates along with *Mougeotia* 11 µm and *Stigeoclonium* . Above pH 4.8 these species are replaced by others, particularly *Mougeotia* 17µm and *Pseudanabaena*, with the species of *Phormidium* and *Oscillatoria* at pH values of 5.2 or above. *Oedogonium* 15 µm was present at the start of the experiment but failed to survive in any of the treatments.

Diatoms were also present on the slides in these experiments but have not been enumerated. The difference in colour of the periphyton developed on the slides suggested however that there might be a shift in the relative contribution of green algae and diatoms with a change in pH, since the biomass developed below pH 5.2 was deep green in colour, while at higher pH it was more brown. Such a shift in dominance might be due to different growth preferences of individual species in both groups, or the increased prevalence of greens at lower pH might be due to decreased competition from the diatoms, as suggested by Stokes (1981, 1986).

The effect of removing the competition by diatoms was tested in the second experiment involving growth of algae in 2 litre tubs, with and without the addition of 5 µg l^{-1} germanium. Ge is a competitive inhibitor of silicon uptake by diatoms and its use has been suggested as a means of suppressing diatom growth in mixed cultures (Thomas and Dodson, 1974). Ostrofsky and Bellmer (1987) however concluded that the effects of Ge were not uniform in different cultural conditions, so that total suppression of diatom growth could not be relied upon for productivity studies, although it is probably a useful tool in preventing the over-

growth of cultures of more slow-growing algae by diatoms. This is essentially what is required in the germanium tub experiment, the lessening of competition from diatoms. In fact very few diatoms were present in the +Ge tubs at the end of the experiment and those seen were probably the remnants of the original population before transfer to Ge.

The relative abundance of filamentous species at different pH, with and without Ge is shown in Table 28. *Hormidium* 5.5 µm is seen to be fairly evenly spread across all treatments, in comparison with tub experiment 1. *Hormidium.* 8 µm, as in the first experiment, was too unevenly distributed for any valid conclusions to be drawn. *Geminella* 8 µm again achieved its greatest abundance around the middle of the pH range, and appears to be somewhat enhanced by the suppression of diatom growth in the +Ge treatments at pH 4.5 and 5.0. This does not appear to occur consistently with any other species. *Pseudanabaena* had an unusual distribution, being common at pH 4.5 and 5.0 in the presence of Ge, and at pH 6.0 in its absence

Stigeoclonium in the Ge experiment grows better at pH values greater than 5.0, in contrast to tub experiment 1. As with the channel experiments, this may be due to a different source of the inoculum.

No cyanophytes were present in the Ge experiment with the exception of *Pseudanabaena*, which appears from all the results obtained in tub and channel experiments to be capable of growth at pH values downto 4.4 . The lower pH limit would appear to be around pH 4.4, but in the tub experiments upward drifts of pH occurred, and frequent manual readjustment to the target pH with dilute H_2SO_4 was required. In consequence the pH values reported are target values and not necessarily the mean values to which the algae were exposed.

One possibly significant difference between tub experiment 1 and the Ge experiment was the lack of temperature control during experiment 1, which is therefore thought to have been exposed to an ambient temperature of 16°C, possibly more during the light period. One consequence of this might be the growth of the Cyanophytes in this experiment. Cyanophytes (= Cyanobacteria) are more tolerant of high temperatures than the eucaryotic algae (Brock, 1973) and even at modest temperatures may acquire a greater competitive edge.

A second consequence, which may be related to the first, concerns the poorer growth of *Hormidium* 5.5 µm at higher pH in experiment 1. This may be due to a reduced availability of D.I.C. to *Hormidium*. In nature this alga is most commonly found in riffles, usually in very shallow, turbulent water or even subaerially at the edge of waterfalls and in splash zones, possibly indicating a preference for high concentrations of dissolved CO_2, and less readiness

to utilize bicarbonate. At higher temperatures there may be less CO_2 available at higher pH than at the lower pH (despite the decrease in CO_2 solubility with decreased pH).

The effect of germanium on biomass developed at different pH is shown in Fig. 24b and Table 30b. In order to enable comparisons to be made between growth in Experiment 1 and Experiment 2, the Ge experiment, biomass has been expressed per litre of medium used.

In the treatments without Ge, both chlorophyll a and carotenoid show a decline between pH 4.0 and pH 5.5, and a subsequent slight rise. In broad terms, a similar decline with pH was seen for chlorophyll in tub experiment 1, but this was not shown by carotenoid. A possible reason for this is the longer period of incubation in experiment 1, which might allow for the accumulation of non-photosynthetic biomass. If this is so, it appears to occur preferentially at higher pH, in contradistinction to the interpretations of reduced heterotrophic activity at lower pH (Hendrey, 1976). Another explanation would be the different taxonomic composition at different pH levels, the green algae abundant at the lower pH having a higher cell-specific content of chlorophyll than the blue-greens and diatoms.

In the shorter term Ge experiment there appears to be no discrepancy between chlorophyll and carotenoid in either + or -Ge treatments. Since it was evident on microscopic examination that diatoms are inhibited by this concentration of Ge , it therefore seems unlikely that the presence of diatoms in experiment 1 would be the explanation for the different distributions of chlorophyll-a and carotenoid seen, and more likely that the blue-greens, with their polysaccharide sheaths and dense mat-forming habit, producing self-shading, are responsible for higher values of carotenoid and AFDW compared to chlorophyll-a.

At pH 5.6 in the Ge experiment, there is a very much larger biomass developed in the presence of Ge, i.e. when diatoms are suppressed. It is extremely unfortunate that there is only one point on the graph showing this response and that the experiment was not repeated, since if the result was verified it would imply that removal of competition by diatoms can result in an increased growth of green algae. Thus treatment with Ge would have the same effect as lowering the pH, at least as far as diatoms are concerned. This does not however take into account the differential effects of pH on the growth of other algal species. The effects of Ge on the growth of green algae are similarly worthy of attention.

Germanium is clearly a useful tool in the study of competitive interactions between diatoms and other algae. The results of these tub experiments require to be confirmed by growth rate determinations.

FIG.24:- TUB GROWTH EXPERIMENTS

(a) INFLUENCE OF pH ON BIOMASS

(b) EFFECT OF INHIBITION OF DIATOM GROWTH

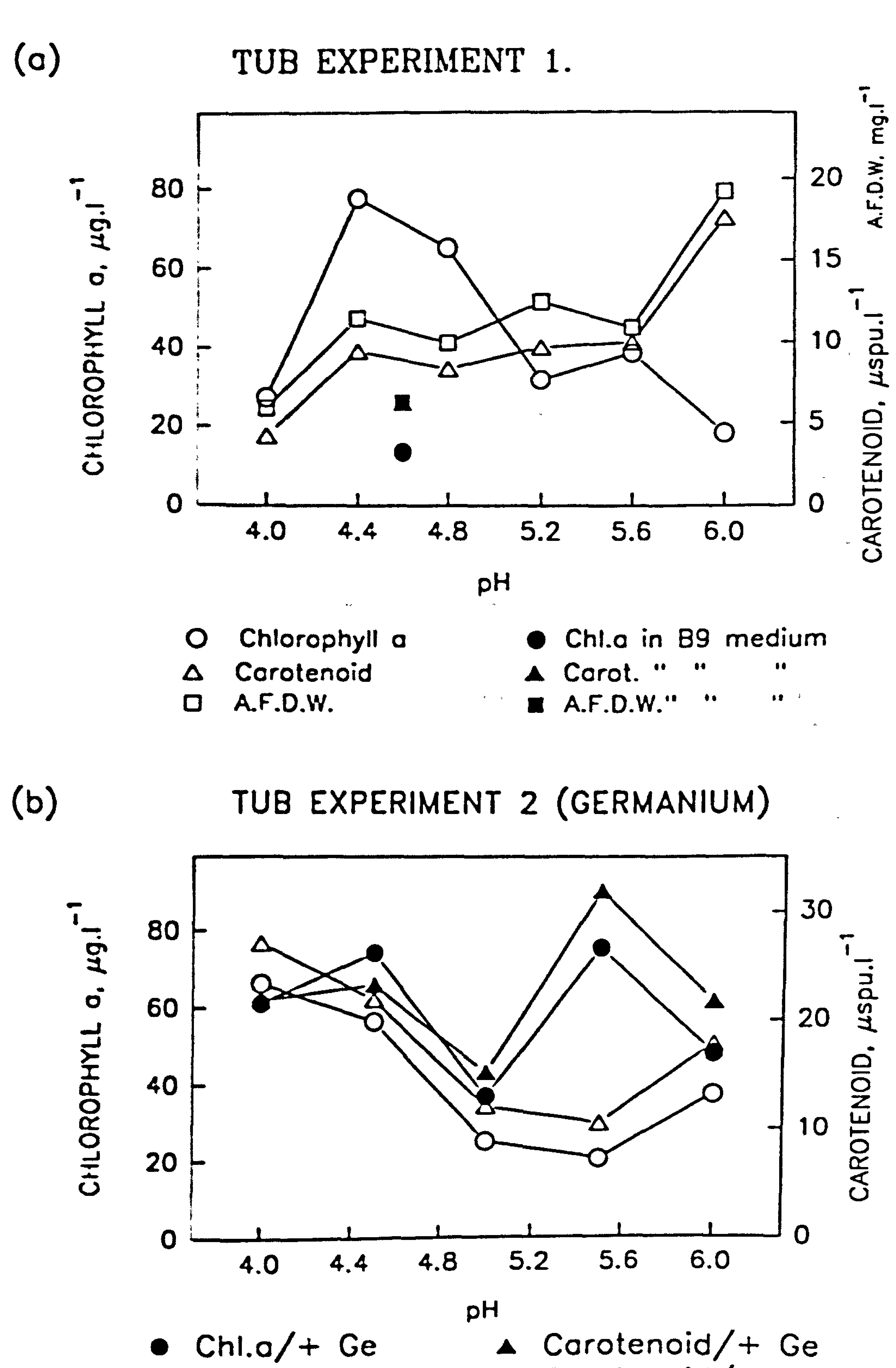


TABLE 28
TUB EXPERIMENT 1. FINAL SPECIES COMPOSITION

Duration: 18 weeks. Vol. of Medium 20 l. Temp. Ambient

Corrie:

	B9	4.0	4.4	4.8	5.2	5.6	6.0
Hormidium 5 µm.	++++	+++	++++	+++	+	++	+++
H 8 µm.	-	-	+	-	+	+	-
Geminella 8 µm.	+	++	+++	++	-	-	-
Mougeotia 8 µm.	++	-	+++	++	+++	+++	+++
M. 11 µm.	++	++++	-	++	++	-	-
M. 17 µm.	++	-	++	+++	++++	++	+++
M. 22 µm.	-	-	+	++++	-	+	-
Stigeoclonium	+++	+++	++++	+++	-	-	++
Pseudanabaena 1.5 µm.	-	-	++++	++++	++++	++++	++++
Phormidium 5 µm.	-	-	-	-	++	++	-
P. 8 µm.	-	-	-	-	++++	+++	++++
Oscillatoria 8 µm.	-	-	-	-	++++	+	-

Other species present at start in all Tubs:

Oedogonium 15 µm.

TABLE 29
TUB EXPERIMENT 2 (GERMANIUM): FINAL SPECIES COMPOSITION

Duration: 11 weeks at 10°C Vol. of Medium 2 1.

MEDIUM:	B9		Corrie: 4.0		4.5		5.0		5.5		6.0	
GERMANIUM:	-	+	-	+	-	+	-	+	-	+	-	+
Hormidium 5.5 µm.	+++	++++	+++	++++	++++	+++	+++	++	+++	++++	++	++++
H. 8 µm.	-	-	+	-	+	-	-	-	-	+	-	+
Geminella 8 µm.	++	++	++	++	+++	++++	++	++++	++	+	++	++
Microspora 8 µm.	++	++	++	++	++	++	+	+	+	+	+	+
Mougeotia 8 µm.	++	+	+	++	+	+	+	++	++	+	++	+
M.. 11 µm.	+++	++	+++	+++	++	++	+++	++	+	+++	++	++
M.. 17 µm.	+	++	++	+	-	-	+	-	+	+	-	-
M.. 22 µm.	++++	++++	-	+++	++	-	+	++	+++	+++	+++	++
Stigeoclonium	++	++	+	-	++	++	++++	++++	+++	++++	++++	++++
Zygnema 20 µm.	-	+	-	-	-	-	+	-	+	-	-	-
Pseudanabaena 1.5 µm.	-	-	-	-	-	+++	-	+++	-	-	+++	-

Other species present at start in all Tubs:

Draparnaldia

Oedogonium 15 µm.

TABLE 30
TOTAL FINAL BIOMASS DEVELOPED AT DIFFERENT pH VALUES

(Expressed per litre of medium used. Single values only, no replicates)

(a) TUB GROWTH EXPERIMENT 1

MEDIUM/pH		Chl.a. $\mu g.l^{-1}$	Carotenoid $\mu spu.l^{-1}$	A.F.D.W. $mg.l^{-1}$
B9	4.6	13.0	6.22	6.25
CORRIE	4.0	27.2	4.24	5.95
	4.4	77.4	9.41	11.45
	4.8	64.9	8.31	9.95
	5.2	31.6	9.69	12.50
	5.6	38.4	9.99	10.90

Conditions: Ambient temperature (~16 °C)
PAR not measured. Fluorescent lighting. 12: 12 h. L-D cycle.
Total volume of medium used (batch replacement) = 20 l.

(b) TUB GROWTH EXPERIMENT 2 (GERMANIUM)

MEDIUM/pH		Chl.a. $\mu g.l^{-1}$		Carotenoid $\mu spu.l^{-1}$	
		+Ge	-Ge	+Ge	-Ge
B9	4.6	6.25	9.85	3.78	4.3
CORRIE	4.0	61.11	66.24	21.91	27.11
	4.5	74.08	56.28	23.24	21.88
	5.0	36.72	24.90	15.16	12.03
	5.5	75.36	20.30	31.90	10.47
	6.0	47.96	37.33	21.65	17.83

Conditions: 10°C
PAR 0.1 - 0.25 $\mu moles\ m^{-2}\ s^{-1}$. 12:12h. L-D cycle.
Total volume of medium used = 2 l.

3.6 CULTURAL STUDIES ON *Stigeoclonium* ISOLATES

Stigeoclonium samples were isolated from the following sites:

(1) Ward burn, sampled 15.6.88
(2) Burn 2, subsample (f), sampled 14.6.88
(3) Burn 6, subsample (c), sampled 14.6.88
(4) Burn 10-2, subsample (a), sampled 14.6.88
(5) Burn 9, subsample (e), sampled 14.6.88

Tests were also conducted on *Draparnaldia* originally isolated from Burn 6.

Growth tests were carried out according to the procedure of Cox and Bold (1966) which involves growth in a flask with a slide half-immersed in the medium. The flasks were bubbled with filtered air, and exposed to 12:12 hour light/dark illumination, 50 µmoles $m^{-2}s^{-1}$ at a temperature of 22°C. After a suitable period of incubation, slides were examined for attachment and development of zoospores.

It was found that only one isolate, from the Ward burn, was able to grow and develop in the Cox and Bold medium. Adjustment of the pH of the $BBMPTB_{12}$ medium was attempted in order to circumvent this problem. Growth checks were made at pH 7.5 (the original pH of the medium), pH 6.0, 5.0 and 4.5. Simultaneous growth checks were made in unmodified Corrie and B9 media. The nutrient content of these media is compared in Table 3.

The results of the growth experiments are summarised in Table 31. The feature of major importance for taxonomy is the morphology of the basal system (Cox and Bold, 1966; Simons *et al*, 1986). Continued examination of the development of isolates was curtailed by contamination by cyanophytes or cessation of growth of some isolates. Only isolates 9e and 10a were maintained in a viable condition and were used subsequently in channel growth experiments.

Based on the data obtained, the isolates correspond to the Simons *et al* (1986) description of *S. tenue*. Germination of the zoospore gives rise to a basal system consisting of initially a single filament, while the erect system grows more rapidly and gives rise to frequent branching. In older specimens a degree of branching of the basal system was seen but not on as extensive a scale as reported for *S. farctum*. The erect filaments seens were never as broad as those reports for *S. helveticum*, although Simons *et al* (1986) reported some specimens only 5.4 to 10 µm in diameter.

TABLE 31
RESULTS OF GROWTH EXPERIMENTS ON *Stigeoclonium* ISOLATES

			Stigeoclonium isolate					*Draparnaldia*
			WARD	2	6	10	9	
MEDIUM	pH							
$BBMPTB_{12}$								
	7.5	a	++++	+	+	-	+	+++
		g	++++	+	+	++	++	+++
		d	7-8	8-11	8-11,14	5	11	8-14;35*
	6.0	a	+	++	++	+++	+	+
		g	+	+++	++	++	+++	+
		d	5	11	8-14	5	8	8-14;30*
	5.0	a	+	+	-	+++	+	/
		g	++	+	++	++	+++	/
		d	5	8	8-11	5	8	/
	4.5	a	-	-				
		g	+-	-	-	-	-	-
		d						
Corrie	>6.0	a	-	+	+++	++	++	+
		g	+	+	+	++	++	+
		d		8-11	8-11	8	11	8-14;30*
B9	4.6	a	-	-	+	++	+	-
		g	-	-	+	++	+	-
		d	-	-	8	8	8-11	
Morphology of basal system			Single long rhizoid 5 µm.	Single rhizoid 5 µm.	Single rhizoid, or sparsely branched 5 µm.	Single rhizoid 3 µm.	Single rhizoid 3 µm.	Single rhizoid 5 µm.

*diameter of differentiated main axis, µm.

a = attachment to slide
g = growth of inoculum
d = cell diameter

The Ward burn isolate is the broadest of those examined and is less similar to the others in its morphology. The early development of *Draparnaldia* resembles that of the *Stigeoclonium* isolates, but its filament is much broader and it grows more slowly, as seen also in channel growth experiments. The main axis differentiation of *Draparnaldia* is reported to be suppressed if the concentration of calcium is less than 1.7 mg l^{-1} (= 86 μeq l^{-1})(Johnstone, 1978) in which case it may be indistinguishable from some *Stigeoclonium* isolates.

Growth of *Draparnaldia* in $BBMPTB_{12}$ or Corrie medium if continued long enough resulted in main axis differentiation. In modified B9 medium, with added calcium, growth was very poor and only at 200 μeq l^{-1} added Ca^{2+} was one example of main axis differentiation seen. Since the Ca^{2+} concentration in Corrie medium is 190 μeq l^{-1} the other nutrients and minerals present must also influence the ability of *Draparnaldia* to undergo main axis differentiation.

In $BBMPTB_{12}$ and Corrie medium there is little likelihood of a healthy, adult plant of *Stigeoclonium* being confused with *Draparnaldia*..

The morphology of the Ward burn isolate sets it somewhat apart from the rest, and it may be a variety of *S. helveticum* Vischer, rather than *S. tenue* (C. Agardh.) Kützing.

Although it is concluded that most if not all of the isolates are *S. tenue*, there are clearly differences between them in their development, morphology, and pH optimum for growth.

Ecotypic variation has been previously recorded for *Stigeoclonium* (Francke and TenCate, 1980; Francke, 1982; Francke and Rhebergen, 1982). This means that identification to species is not necessarily sufficient for indicator organisms. If the types are morphologically distinguishable they may be scored separately and the data used whether or not these types belong to different species. As a corollary, if two species are morphologically indistinguishable in field samples, their indicator value is diminished. In either case it is important to check the growth characteristics of field isolates in laboratory conditions.

4.0 DISCUSSION

4.1 CHARACTERISTICS OF SITES

Because of the requirement of photosynthetic organisms for light, attempts were made when choosing sampling sites to find as open a location as possible. Nevertheless, with much of the sampling area being under forestry, considerable differences between sites exist, as reflected in the measured values for PAR as a percentage of that at the reference site at burn 6. The light sensors were mounted near water-level within the stream bed, but differences will occur at different points up, down and across the stream due to bank shading, etc. It is therefore appropriate to treat the data obtained as a rough guide for comparisons between sites and not as an indication of the general availability of light to the rest of the stream.

The degree of insolation however has an influence on the temperature of the stream water. If discharge, depth, altitude (hence air temperature; Webb and Walling, 1986), and substrate type are the same, a stream with an open course will have higher day time and lower night time temperatures than a stream running within forest (Weatherley and Ormerod, 1990). It should be possible therefore to calibrate a given stream so that the temperature difference between two points could be related to the amount of light incident on that reach of the stream (Wong *et al*, 1978).

Some differences are usually found between upstream and downstream sites on the same stream, i.e. on burns 10, 11 and 14. The pairs of sites on these streams are a short distance apart (about 400 m or less) and there are no major inflows of water between them.

The course of burn 14 between the upstream site 14-2 , and 14-1 downstream, is a mixture of open course, a canalised section flowing over a bed of cobbles, and a series of cascades within coniferous forest. Both sites lie within a pylon line clearing in the forest. The mean temperature difference between the two sites is + 0.18°C (Matrix B).

Prior to clearfelling, the entire course between 10-1 (upstream) and 10-2 was moderately to densely shaded by coniferous forest. The mean temperature difference is + 0.14 (Matrix B).

Between 11-1 (upper) and 11-2, the stream crosses a pylon line clearing, enters a narrow gorge and reenters the forest where it plunges over a waterfall, dropping approximately 30 m. in total. The mean temperature difference is -0.06°C (Matrix B).

Burns 5 and 6 have a high mean temperature (about 11°C) as their courses are still relatively open. At site 5/6 some 200 m. below their confluence, the mean temperature has risen further to 14.5°C. However 5/6 has been sampled less frequently than the main sites 5 and 6 and therefore the difference of 3.5°C may be biased. Nonetheless the trend is clear, and the potential exists to exploit these temperature changes if it is desired to estimate the amount of PAR received by a given reach of a stream. Estimates of percentage incident PAR have been made for sites 14-1, 15, 5/6, 10-2, 11-2, C-1 and Ward.

Besides light availability, sites differ in discharge characteristics and substrate type, which depend on the geological and topographical characteristics of the catchment. Soil and vegetation type also influence discharge and water chemistry. Burn 14 drains a predominantly forested catchment and runs within forest for part of its course. The major part of the course however is within broad clearings. The upper site, 14-2, has a substrate consisting of bedrock which forms a series of cascades. The gradient of the stream is moderate so that considerable spates may occur, during which some smaller stones are mobilized. However, in the upper reaches of the stream, the tributaries show evidence of quite stable beds, having mosses attached to stones as small as 5 cm diameter. At the downstream site, 14-1, there was little problem encountered with stones damaging slides, or of silting on the slides, although some problems with drifting vegetation occurred. The water is moderately humified, and has a mean pH around 5.0.

Burn 15 drains a steep, south-facing slope which was recently planted with conifers but has considerable natural deciduous bankside trees. Its quality was previously good as it still retained a fish population at the time of the field work. Whether this will remain the case is uncertain. The site is next to the public road, just downstream of a road bridge and is quite heavily shaded by bankside trees. The substrate type is quite large stones, approximately 15 cm. or more in size, since very large discharges of water occur during spates. There is little evidence of silting occurring. No artificial substrates were used at this site. The water is very clear.

Burn 2 drains an almost totally treeless catchment, and is the control for the effects of afforestation within this group of burns utilized by the Freshwater Fisheries Laboratory, Pitlochry. It drains a large area on the north slopes of Ben Lomond and is subject to very large discharges during rainy weather. The substrate type is cobbles 20 cm or more in size, with some bedrock, but considerable deposition of silt and fine gravel also occurs during decreasing flows.

The potential for substrate movement and abrasion is therefore considerable. The water is slightly humic, with quite low levels of Al, and supports the presence of fish, although the mean pH is only 5.3.

Burn 5, like 6, drains a large moorland catchment before entering a fairly young forest. The substrate type is quite small pebbles of shale, with some bedrock. The shale is easily disturbed during a spate and considerable abrasion and loss of slides was encountered at this site. There is little vegetation shading the stream, but the banks at the side are quite high. This stream supports few if any fish. Water quality parameters are similar to those of Burn 2.

Burn 6 has a more stable substrate type than 5. Shale is present but a larger proportion of the substratum consists of cobble or boulder. Flow over these can be quite turbulent and some mobilization and deposition of small stones can occur but losses of slides at this site were rare. There is little moss or liverwort growth apparent, which might signify an abrasion problem, but some algal mats were of quite long-term duration, notably growths of *Tolypothrix* and some longer-lived Rhodophytes such as *Lemanea* and *Batrachospermum* were found.

Burn 5/6 shares some of the characteristics of its tributaries. The substrate type is fairly large cobbles or boulders, implying that smaller stones are moved downstream during spates. However a considerable amount of *Fontinalis* moss grows in this stream, together with a semi-permanent population of *Batrachospermum* at the sampling site, showing that shading against current and abrasion must occur. Fish occur in 5/6.

Burn 9 has one of the lowest mean pH values of all the streams. It drains a forested catchment with deep peat deposits, and the water is highly humic. It is also subject to considerable spates during which small pebbles and stones are mobilized. This may be partly a consequence of forestry activities occurring upstream, where a ford is used by heavy machinery. Losses of slides and breakage of tiles have occurred, as well as the more usual problem of abrasion. Siltation as such has not been a problem. The site lies on a straight reach lying between high ground on either side. Midday values for PAR will be high as the course runs S-N, but shading during the earlier and later parts of the day reduce the total incident PAR to 70%. Mean values of Al are similar to burn 14.

The burn 10 catchment lies wholly within the forested area. The substrate is mainly pebble with a few cobbles. Considerable problems were encountered with abrasion and loss of slides, and siltation on tiles. During a dry period this burn was reduced to virtually zero flow, much lower than any of the others. Its catchment area is one of the smallest. The gradient between the two sites is very slight. Low light level was one of the factors presumed to be reducing the amount of algal growth seen at sites 10-1 and

10-2. It remains to be seen what the effect of bankside clearance will be. Immediately following clearfelling there was too much disturbance, sedimentation and obstruction by brushwood for any effect on algal growth to be manifest. Previously the only large development of algal biomass which could be seen was of *Hormidium* sp. on stones below the measuring flume, where a constant splashing occurred. The water is quite low in humic material (A_{250} < 0.3) but has the highest recorded mean Total Monomeric Aluminium (Al-TM) and Labile Aluminium (Al-L) concentrations. The mean pH is higher than that of burns 9 and 11, at about 4.6 in Matrix B.

The burn 11 catchment also lies totally within the forest, but the catchment characteristics are somewhat different from Burn 10. Burn 11 rises from a peaty bog and descends little before site 11-1. In consequence the flow regime at this site is quite mild. The banks are peat, the substratum is boulder with small pebbles and gravel. The boulders and bedrock emerging downstream before the waterfall previously alluded to, support a luxuriant growth of moss and liverwort. There is usually little algal biomass found on either natural or artificial substrates. A fine deposit of organic material may usually be found on substrates. The A_{250} is high (mean > 0.6). The mean pH is marginally lower than burn 9 in Matrix B (1987-88). Al is lower than in burn 10. Al-L concentration is similar to that in burn 9.

Site 11-2 is subject to more abrasive discharges and breakage or loss of slides was encountered upstream of the gauging flume. Downstream of the flume there was less abrasion but high current velocities occasionally caused losses. Low light levels due to shading by the trees growing within 2 m of the bankside reduced the amount of algal biomass found in the stream, but high densities of algae and moss occurred on the apron of the flume, presumably because of the highly stable surface and the presence of a sediment trap upstream of the flume.

The Corrie burn lies wholly within the forest but has four tributary streams which straddle the Highland Boundary Fault. Measurement of pH in these tributary streams (Kinross, 1985) showed a rise in pH from west to east. The acidified burn 9 (Kelty water) catchment lies adjacent to that of the most westerly (lowest pH) tributary of the Corrie. The three most westerly tributaries unite upstream of the upper Corrie site (C-1). The fourth, highest pH, tributary joins the main stream about 200 m downstream of C-1. It however contributes a very small volume of flow to the burn.

The upper site is heavily shaded by coniferous forest approximately 30 years old. The gradient upstream is quite steep and a great deal of abrasion takes place by sand and gravel during spates. Bricks with slides attached were regularly washed downstream and a tile rack

silted over. The substratum consists of cobble and boulder, deposits of finer material and some exposed bedrock. Moss occurs on some larger boulders. Fish occur in this stream. The water is clear, with low levels of Al (Al-TM < 55 $\mu g l^{-1}$), and a mean pH (Matrix B) of 6.25). Possibly because of the current regime, large accumulations of biomass were not normal.

The downstream site C-2 changed location between 1986 and 1987 seasons because of severe erosion which shifted the course of the stream, burying a tile rack under 1 m. of gravel.

Some differences in algal taxa recorded may occur because of this change. A large protrusion of bedrock had previously supported a growth of *Batrachospermum* , which was therefore always recorded as being present at this site. Following the erosion the rock was covered and the site characteristics changed to a bed of stones < 10 cm diameter.

The post-1987 location was about 400 m downstream, at a bend with bedrock, boulder, pebble and gravel present. Loss of slides and tiles did not occur at this site but there was considerable deposition of fine silt and organic debris. The lower part of the catchment, between C-1 and C-2 sites, was undergoing a clearfelling operation during this period, along the eastern bank.

Around the post-1987 C-2 site clearfelling had already occurred, allowing greater light penetration (60% of burn 6) than would have existed previously, and greater than would have existed at the pre-1987 location. The lower Corrie burn supports a fish population. The mean (Matrix B) pH is 6.5 (spot) to 6.8 (composite sample), approximately 0.2 units higher than at the C-1 site. The water is clear and levels of Al the lowest of all the streams sampled. Concentrations of silica are the highest found in those streams assayed. Bedrock supports a permanent population of *Lemanea.*

The Ward burn was chose as an alternative 'control' site to the Corrie burn when it was feared that the felling operations on the latter might cause problems. However the Ward burn is considerably different from the Corrie. It has a mean pH which is slightly higher, but is a very much smaller stream arising in a boggy area and draining a forested, peaty catchment smaller in area to the Corrie. The total discharge of the Ward burn is therefore very much less. However the stream runs between steep narrow banks and high flows in spates cause bed instability. Loss of slides due to damage by stones and siltation of slides and tiles occurred. The water has a higher mean A_{250} than even Burn 11, but higher Al values than the Corrie burn. The substratum is a mixture of bedrock, cobbles and stones. A permanent colony of *Lemanea* is present on some of the bedrock.

4.2 SAMPLING METHODS FOR SPECIES AND BIOMASS

The highly heterogeneous nature of the environment found in the upland streams studied presents particular difficulties in quantitative sampling.

Diatoms may be sampled from a determined area of substrate using special apparatus (Douglas, 1958) but larger filamentous algae have a markedly patchy distribution (Butcher, 1941) which could only be effectively sampled by very intensive quadrat methods. Such a sampling programme would seriously limit the scope of the investigation while possibly contributing little in additional information (Fryer, 1987). In addition the enumeration of the species present in such samples presents great practical difficulties as the sample cannot be divided into aliquots for counting unless it is first homogenised or sonicated to break up clumps, which would destroy some of the characteristics necessary for identification e.g. in the genera *Tolypothrix*, *Stigeoclonium* and *Draparnaldia*.

For this reason a very simple sampling procedure was adopted, the aim of which was to obtain representative samples of algal growth across the whole range of environmental niches, current regimes and substrate types found at a particular site on each stream and to enable as wide a range of species to be detected as possible. A similar procedure was adopted by Jackson *et al* (1990), picking samples of representative growths and estimating percentage contribution to biovolume from replicate samples and by Say and Whitton (1980) and Morgan (1987). Frequent sampling of the same habitats may assist in the identification of difficult specimens (Hillebrand, 1973).

Attention was directed to filamentous algae as these have been noted to apparently respond positively to acidification (Muller, 1980; Arnold *et al*, 1981; Stokes, 1981, 1986) and were therefore considered as possible biological indicators. However their filamentous nature presents further difficulties in determining the quantity of each species in a sample. Because of this difficulty and the additional problems of quantitative sampling already referred to, no attempt was made to actually enumerate species abundance but rather to rank them in relative abundance. Any attempt to use this data to determine species covariation or covariation between species and environmental variables must therefore be treated with caution.

The best measure for biomass is open to interpretation. Chlorophyll content is widely used but it varies with light intensity, nutrient availability, physiological state, and species. Carotenoid has been advocated as a better correlate with algal volume (Foy, 1987) but this work was carried out on an essentially unialgal population. Dry weight or ash-free dry weight has the disadvantage that it may include animal biomass and allochthonous material, as well as photosynthetically inactive algal material and bacterial biomass in dense mats.

4.2.1 Species Identification Problems

Filamentous algae are notoriously difficult to identify to species level. Early workers described each variation found as a separate species, ignorant of the amount of phenotypic variability shown by many. This situation is well illustrated in the genus *Stigeoclonium*, as reported by Cox and Bold (1966).

The genus was originally described by Kützing (1853); in 1963 Islam reviewed the literature on the genus and noted reports of 100 different species, providing descriptions of the morphology of a smaller number of species. However Cox and Bold (1966) found that *Stigeoclonium* species were polymorphic for many of the characteristics of the erect thallus utilised for identification by Islam (1963). They examined 100 isolates and reduced them to a list of 20 which were then cultured under standard conditions. They concluded that the morphology of the basal system was a more reliable attribute for species identification, which allowed them to reduce the final list to six species (not necessarily an exhaustive list however).

This methodology was extended by Simons *et al* (1986) who reduced to three species the examples of the genus found in the Netherlands. However it has not been adopted by all recent workers, e.g. Gibson and Whitton (1987).

Attempts to utilize the methods of Cox and Bold (1966) have failed in this study because only one out of four isolates tested was able to grow under the 'standard conditions' of Cox and Bold. It was found that the pH of the medium had to be reduced in order to allow growth of some isolates.

Polymorphism in *Stigeoclonium* may be due to genetic differences between populations where exotypes exist adapted to environmental variables, e.g. chloride (Francke and TenCate, 1980; Francke, 1982; Francke and Rhebergen, 1982), zinc (Harding and Whitton, 1976; Say and Whitton, 1980), or due to morphogenetic plasticity, as a response to environmental conditions, for example the formation of hairs in response to deficiency of PO_4^{3-} or NO_3^- (McLean and Benson-Evans, 1977;Whitton and Harding, 1978; Gibson and Whitton, 1987).

Other genera have been shown to exhibit ecotypic variation related to environmental conditions, e.g. the response of *Hormidium* species to zinc (Say *et al*, 1977a; Say and Whitton, 1980).

The possible existence of strains differing in their tolerance to environmental variables, for example pH, reduces the likely value of such a taxon as an indicator organism, unless the tolerance level is identified by laboratory tests for each population.

In view of the polymorphism of the erect thallus, field specimens cannot be readily identified to species. Indeed there is room for confusion of *Stigeoclonium* species with the related genus *Draparnaldia*, which is also reported to be polymorphic. *Draparnaldia* is reported to show *Stigeoclonium* - like morphology under conditions of low calcium concentration (<1.7 mg/ml) (Johnstone, 1978 a,b; Van Beem and Simons, 1988)

Van Beem and Simons (1988) examined the growth of *Draparnaldia mutabilis* in synthetic medium and concluded that herbarium specimens and fresh material collected throughout the Netherlands could all be ascribed to the one species. They also report that some *Stigeoclonium* species show *Draparnaldia* -like growth in eutrophic waters, and Johnstone (1978a) reports *Cloniophora* -like growth of *Draparnaldia* under conditions of high nitrate concentration.

Even more confusingly, Cox and Bold report that *S. helveticum* exhibited many characteristics of the genus *Ulothrix* .

This inability to ascribe a species name to a field specimen is likely to be common to other genera, especially if the observation of sexual reproductive structures is required, as in the Conjugales. For this reason genus names only are used here, plus an identifying characteristic, usually cell diameter, although this itself is variable between close limits, e.g. in *Mougeotia*, or within much wider limits in the irregular tapering filaments of *Oedogonium* , and is not a valid taxonomic character in *Zygnema* (Miller and Hoshaw, 1974).

As indicated above, confusion between genera is also a possibility. In particular, degenerate forms of *Geminella* 8 µm. and *Microspora* 8 µm. have been observed in this study which resemble the described genus *Binuclearia* (Prescott, 1970). These always occur in locations where *Geminella* and/or *Microspora* are frequent, and often occur in the same sample. Therefore it is doubtful whether *Binuclearia* occurs within the Loch Ard area. However in some cases it may not be possible to decide whether a particular filament is *Geminella* or *Microspora*. These filaments show contraction of the chloroplast and thickening of the cell wall or sheath sometimes with a lamellate appearance. The H-sections of the *Microspora* cell wall are not distinguishable under these circumstances, and this may be the only feature which would make identification possible.

Problems may also arise within the Cyanobacteria if only small pieces of filament, or hormogonia, of e.g. *Tolypothrix* occur in a sample. These may be erroneously ascribed to the genus *Lyngbya*, but might equally be *Plectonema* or *Scytonema* if the mode of branching is not evident. Similarly *Oscillatoria* may be confused with *Phormidium* if motility is not observed.

Notwithstanding these problems some tentative identifications are presented with a description of each taxon in Appendix 1.

4.2.2 Estimation of Biomass

Spectrophotometric analysis of chlorophyll has been widely used as a convenient method for estimating algal biomass, or biovolume. However there are uncertainties involved in the extraction processes; different workers using different organisms have produced diametrically opposed recommendations as to method.

The recommendation of Strickland *et al* (1968) and Parsons *et al* (1984) is that 90% acetone should be used as an extractant, $MgCO_3$ should be used in the filtration, and that grinding or maceration of samples is helpful, while freezing filters leads to low extractability.

Marker (1972), Holm-Hansen and Riemann (1978), and Marker *et al* (1980), recommended methanol due to the poor extraction of pigments from green and blue-green algae by acetone, and report a loss of pigments due to storage of filters frozen in the presence of $MgCO_3$, but found that grinding is not necessary if methanol is used as the extractant.

Sartory and Grobelaar (1984) found methanol or ethanol superior to acetone or acetone/DMSO for the extraction of pigments from phytoplankton. However Hansson (1988) found acetone superior to methanol when used with sediment samples, although 96% ethanol gave similar results to 90% acetone, and freeze-drying of the samples improved the yield. Wood (1985) found chloroform-methanol superior to all other extractants with various algal samples.

Chlorophyll may be naturally degraded to phaeophytin and this may be estimated from the change in absorbance following acidification; this converts the remaining chlorophyll to phaeophytin, which has a lower extinction coefficient than chlorophyll. Thus the phaeophytin content may be estimated from the change in the absorbance peak. If the extract is in 90% acetone, the absorbance may simply be read at 663 nm before acidification with HCl, and after at 665 nm (Marker, 1972) but in methanol the absorption coefficient is pH-dependent, so that the extract requires to be neutralized following conversion to phaeophytin (Marker, 1972, 1977; Marker *et al* , 1980) making the

treatment of large numbers of samples more laborious.

For these reasons acetone was initially utilized as the extractant though without maceration of filters as my own findings were that this was not necessary (Kinross, 1985). However a variable degree of pigment extraction was evident with some of the field samples, and slides with cultured *Hormidium* sp. in particular were found to give very low extraction of chlorophyll-a, as judged by visible colour remaining in the algae after extraction. Also the estimation of phaeophytin in replicate samples was found to give variable results, possibly due to an ineffective acidification procedure. For these reasons, 90% methanol, which effectively extracts a high percentage of chlorophyll-a from *Hormidium* and other cultured species, was adopted instead of 90% acetone. The estimation of phaeophytin was discontinued owing to the greater amount of work involved with methanol extracts (Marker, 1972). Chloroform-methanol extraction (Wood, 1985) was used unsuccessfully on *Hormidium* . Ethanol is reported to be as efficient as methanol (Jespersen and Christoffersen, 1987) and is less hazardous in use. The trichromatic method of Parsons and Strickland (1963) was used to calculate chlorophyll -a concentrations in both acetone and methanol extracts; transfer from one solvent to another by the method of Marker (1972) showed that there was effectively no difference in absorbance of extracts in the two solvents (Table 32).

It is concluded that chlorophyll-a may be calculated using the trichromatic equations from absorbances measured in either 90% acetone or 90% methanol. Carotenoid is underestimated if calculated from A_{480} measured in 90% acetone. Foy (1987) showed that carotenoid measured in methanolic extracts of phytoplankton was a better estimator of algal biovolume than chlorophyll-a, though it should be noted that he was working with samples composed mainly of species of *Oscillatoria* (Cyanophyta).

These results appear to indicate that there is little overall difference in the suitability of chlorophyll-a or carotenoid as an estimator of algal biomass. For theoretical reasons it may be supposed that carotenoid might, as Foy (1987) finds, be more reliable, as the specific content of chlorophyll varies with a cell's exposure to light and the nitrate content of the medium (Round, 1973).

TABLE 32
COMPARISON OF PIGMENT ABSORBANCES
MEASURED IN ACETONE AND METHANOL

Pigments extracted in 90% MeOH, divided into 13 aliquots, 10 dried under reduced pressure at 30° in rotary evaporator (Marker, 1972), taken up in 90% acetone.

MEAN (S.D.)

	n	$A_{663/4}$*	A_{480}	Chla µg (trichromatic)	Carotenoid µspu
in MeOH	3	.404 (.006)	.420 (.004)	53.97 (.83)	2.10 (.01)
in Acetone	7	.413 (.008)	.348(.010)	54.84 (1.06)	1.76 (.05)
" " (double volume dried)	3	.810 (.011)	.674 (.012)	53.83 (.66)	1.69 (.03)

*Methanolic extracts are read at 664nm as the peak is shifted (Marker, 1972).

TABLE 33
CORRELATIONS BETWEEN BIOMASS ESTIMATES FROM SLIDES

Correlations (5% significance level)

Slide correlations:	n	AFDW/Chla	AFDW/Carot.	Chla/Carot.
Overall	95/65/65	0.227 (0.20)	0.244 (0.24)	0.952 (0.24)
Nov. 1987	21	0.786 (0.43)	0.959 (0.43)	0.989 (0.43)
June 1988	15	0.414 (0.51)	0.660 (0.51)	0.919 (0.51)

Ash-free dry weight of lab-grown algae is the best measure of their biomass, notwithstanding possible inaccuracies in measurement due to:

(a) insufficient drying at 60°C
(b) uncertainty in weighing
(c) loss of material after ashing
(d) increase in weight after drying and ashing by uptake of atmospheric water vapour
(e) loss of weight due to the presence of carbonates.

Inaccuracies due to a, c, d and e may be minimised by careful procedure, but the inherent uncertainty in weighing, estimated as ±0.1mg, may form a considerable proportion of the total AFDW in some cases. The accuracy of AFDW determination, in absolute terms, is also affected by the combustion temperature (Ridley-Thomas *et al*, 1989).

In the case of field samples, additional inaccuracy may result from the presence of allochthonous material, detritus, animal or bacterial biomass, so that poor correlation of pigments with AFDW may be expected. Selected sets of results from slides removed in November 1987 and June 1988 show high and low correlations respectively between AFDW and pigments, suggesting seasonal variation in the presence of such non-algal material on the slides (Table 33). In such cases pigments may be the only suitable measures of algal biomass (Hillebrand, 1973).

The other parameter of algal abundance frequently measured is biovolume, but this requires (a) cell counts and (b) measurements of critical cell dimensions enabling a theoretical calculation to be made (Clarke *et al*, 1987). In this work no attempt has been made to perform cell counts on field samples for practical and theoretical reasons (Hillebrand, 1973).

The habitat is extremely heterogeneous with respect to flow rate, exposure to light, exposure to abrasion, supply and removal of dissolved gases, nutrients and waste products. Hence it is not surprising that benthic algae have a very uneven distribution (Butcher, 1941; Round, 1981; Robinson, 1983; De Nicola and McIntyre, 1990 (a), (b)). This gives rise to errors in estimation of areal biomass which are usually larger than those due to errors in pigment measurement (Tett *et al*, 1975).

To estimate biomass present in streams, two strategies have been adopted. The first is to sample natural substrates (stones) present in the streams, selecting two (within limits of dimension set by the requirements of the extraction process) which represent the range of periphyton density likely to occur at that site.

The second is to plant artificial substrates which are removed at intervals to sample the periphyton. Two types of substrate were used: 15cm square glazed ceramic tiles, and standard (2.6 x 7.2cm) microscope slides. The slides are fragile and thus frequently lost during spates, and in addition sample a relatively small area (20cm^2), but a larger number can be located at each site. The tiles sample a larger area (225cm ^{2}) and are rarely lost in spates, but a smaller number can be utilized at each site. In practice most sites had 3 tiles and 6 slide pairs; some had 12 slide pairs.

These numbers are insufficient to truly sample the sites in a statistically valid way; as with the natural substrates they merely give an indication of biomass present. Most tiles when sampled were 3 months old; the situation with slides is more complex due to frequent losses. Slides were also more difficult to remove from the racks in times of high flow and so were more frequently omitted from the sampling routine at such times. The stones however had been present in the streams throughout: the age of their periphyton would depend on the time of the last spate which was sufficiently vigorous to scour them clean.

Artificial substrates require an initial colonization period, after which the biomass increases exponentially. Herder-Brouwer (1973) found a colonization phase of 10 days, followed by a growth phase up to approximately 40 days. Hamilton and Duthie (1984) found that colonization was logarithmic over a period of 25 days. Lamberti and Resh (1985) found that tiles accurately reflected natural substrates in respect of chlorophyll-a, bacterial and macroinvetebrate density after 28 days, and after 63 days in respect of phaeophytin and AFDW, which would imply that the periphyton had reached a mature state. They recommend an exposure time of 1-2 months. By these estimates, exposure times of 3 months ought to permit the periphyton on tiles and slides to match that on natural substrates.

Meier *et al* (1983) found variability between replicates of less than 8% with respect to chlorophyll a on artificial substrates, the smallest areas used being approximately 2cm^2 coverslips, at exposure times in excess of 10 days. Experience of the visually evident differences present on slides and tiles in the Loch Ard streams would suggest that variability of biomass is in excess of this figure.

Measurement of chlorophyll-a extracted from 6 replicate slides exposed for 4 weeks in an earlier study (Kinross,1985) gave a mean of 7.42 $\mu g.cm^{-2}$ and a s.d. of 1.17; the spread of values was 40% of the mean. Therefore the agreement between a limited number of replicates is not expected to be high.

The major advantages of using slides are that microscopic examination of closely adherent periphyton is possible (some small adnate species were never found in samples brushed off substrates but were noted on the slides) and that pigments and AFDW can be estimated by direct extraction followed by incineration of the growth on whole slide. The slide can finally be mounted for diatom examination.

Tiles have an advantage over stones in presenting a uniform smooth surface which can be thoroughly brushed or scraped clean; the white glaze helps in assessing when all periphyton has been removed. The colonized area is more readily calculated: the area lost at the edges where the plastic channel grips the tile is compensated by the leading and trailing edges where periphyton can colonize the sides.

Extraction of whole tiles after brushing off the periphyton showed that very little biomass occurs on the underside or is left on the top surface after brushing. Slides however are colonized on both upper and lower surfaces, so slides are used in pairs, back-to-back, mounted horizontally. In the calculation of biomass per unit area, the area of both upper and lower slide surfaces has been considered together, as $20cm^2$.

When a high density of algal biomass has developed on the slides the effective surface area for photosynthetic biomass is only half this. There is therefore some ambiguity about the estimates of algal biomass on slides.

Artificial substrates have been criticised as aids to estimation of natural communities (Tippett, 1970), but most particularly in comparison with epiphytic communities. The main problem appears to be that a long period of exposure is necessary for the communities to resemble those present on natural substrates (Herder-Brouwer, 1973; Lamberti and Resh, 1985; Hamilton and Duthie, 1984). This may be because the even surfaces (which are a major advantage of artificial substrates) do not provide the optimum niches for rapid attachment and development of some species (Hamilton and Duthie, 1984). The main advantage is that the artificial substrates provide a uniform surface for colonization in all locations, enabling a comparison to be made between streams with natural substrates of different geological nature (Castenholz, 1961; Klap-wijk, 1978).

However in practice although the surface of the tiles may be uniform, the conditions in which they are presented for colonization are difficult to match perfectly between streams, because of constraints such as the ability to drive the anchoring stakes into the stream bed. This sometimes results in the rack not being aligned with the current. Indeed the pattern of flow in the bed may change with discharge, or may be altered permanently due to bank erosion.

Robinson (1983) recommends replication of primary sampling to reduce variance in the results. The constraints just mentioned make it difficult to arrange multiple artificial substrates at each site, when so many sites are being monitored on a regular basis. Meier *et al* (1983) found that after 10 days of colonization, variability of chlorophyll on coverslips was about 7% with three replicates. However the area sampled in this manner is so small that serious doubt exists whether the periphyton in the Loch Ard streams would be adequately sampled. Colonization of surfaces such as slides and tiles occurs in an uneven manner, initial colonization occurring at the edges (Hamilton and Duthie, 1984; and personal observations). Thus after short colonization periods (less than 25 days, Hamilton and Duthie, 1984) the areal biomass will be dependent on the circumference : area ratio of the substrate.

This 'edge effect' may be due to the disruptive effect of the edge on current flow (De Nicola and McIntyre, 1990a), which might indicate that other interferences with flow may also affect the pattern of colonization on substrates, for example the racks used to retain the substrates. It may be that the stream used by Meier *et al* (1983) for their experiments had sufficient spatial homogeneity to ensure that replicate substrates at different points had essentially the same periphyton density. Nevertheless there is no assurance that the densities observed are truly representative of those on the natural substrates (Robinson, 1983).

Marker and Willoughby (1988) found that the biomass on natural substrates was proportional to the surface area. In this case stability and freedom from scouring may be the causative factors. On this basis the best comparative estimates of biomass may be obtained from tethered tiles.

4.3 EVIDENCE OF SEASONALITY

Seasonal cycles in the values of environmental variables, species abundance, and biomass may be obscured rather than revealed by correlation analysis between them and a time variable, since correlation analysis presumes a linear relationship. Correlations calculated between variables and annual week, squared annual week or square root annual week are low and not significant at 1% (not shown). A plot of temperature against annual week number shows a clear seasonal cycle (Fig. 25a). PAR shows a similar trend, also peaking about week 28. Absorbance data also shows a seasonal peak, around week 35, but with greater scatter of points.

If a seasonal cycle in species abundance or biomass exists it may be revealed via correlation with temperature rather than with annual week.

Differences between mean temperature for different sites are largely an artefact due to samples being taken in a regular sequence, as shown by a plot of mean site temperature against time of sampling (Fig. 25b). Therefore scatter in the temperature data may obscure the strength of correlations in the total data. Seasonality has been investigated by correlation analysis within individual site data sets and in the full data set (Table 34).

Significant correlations (at 1%) are rare. A large positive r value with PAR is to be expected. Some significant positive correlations with absorbance and negative with TON are also found. An increase in A_{250} indicates an increased concentration of humic material, which may occur in summer as a result of reduced dilution and release of humics due to cracking of peat beds and increased microbial activity (Rees *et al*, 1989).

TON may decrease in summer as a result of increased nutrient uptake by periphyton. An increase in TON in winter occurs due to the breakdown of plant material at the end of the growing season. The concentration of other chemical variables is liable to increase during low flow simply as a result of a decreased dilution.

Evidence for a seasonal trend in biomass is scarce. Only one significant correlation with temperature is found, for stone carotenoid in site 14-1. This is unexpected, but may be partly due to the scarcity of data for some biomass measures. Observation suggests that biomass is reduced during autumn spates, but the evidence from measurements is against this during the period of study. Observation again suggests that it is only very strong current and abrasion by suspended material which reduces the quantity of periphyton, and if low-flow conditions occur during winter this 'seasonal' reduction may not take place.

Loss of biomass may also occur following summer rainstorms, and the loss may be greater following a period of uninterrupted growth due to the increased length and drag of the filaments (Horner *et al*, 1990). Nutrient depletion following a bloom may result in more fragile filaments and hence an increased likelihood of breakage. These factors may all militate against a clear seasonal trend in biomass.

Some significant correlations (at 0.1%) between species abundance and temperature are found (Fig. 26) but none has a higher value than 0.341, indicating that a strong seasonal effect on individual species relative abundance does not exist. The species showing the highest values of r, with some other lower correlations, have been plotted in Fig. 26. A second-order regression line has been fitted, and appears to be significant at 0.1% in the case of *Draparnaldia*, *Mougeotia* 8 µm, *Mougeotia* 11 µm, *Mougeotia.* 16 µm, *Microspora* 8 µm and *Hormidium subtile.* However the seasonal effect on abundance is slight in comparison with the amount of scatter in the data, and the actual values of r are less than 0.3. Overall, there is little evidence in this study for seasonality of periphytic algal abundance at either the species or community level.

FIG.25a:-

SEASONAL VARIATION IN FIELD TEMPERATURE;
INDIVIDUAL VALUES FOR ALL SAMPLES
99% C.I.

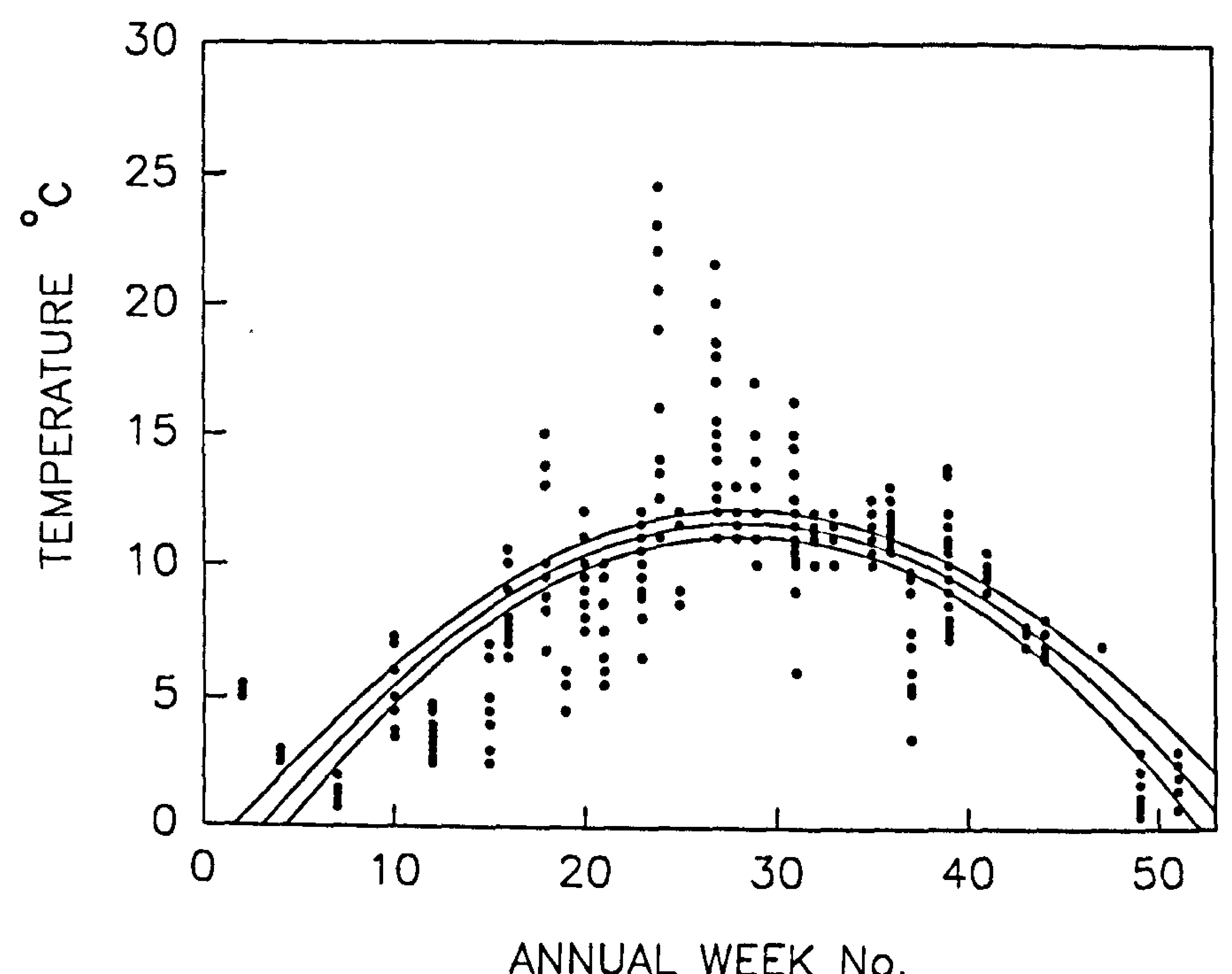


FIG.25b:-

RELATIONSHIP BETWEEN MEAN FIELD
TEMPERATURE AND TIME OF SAMPLING
95% C.I.

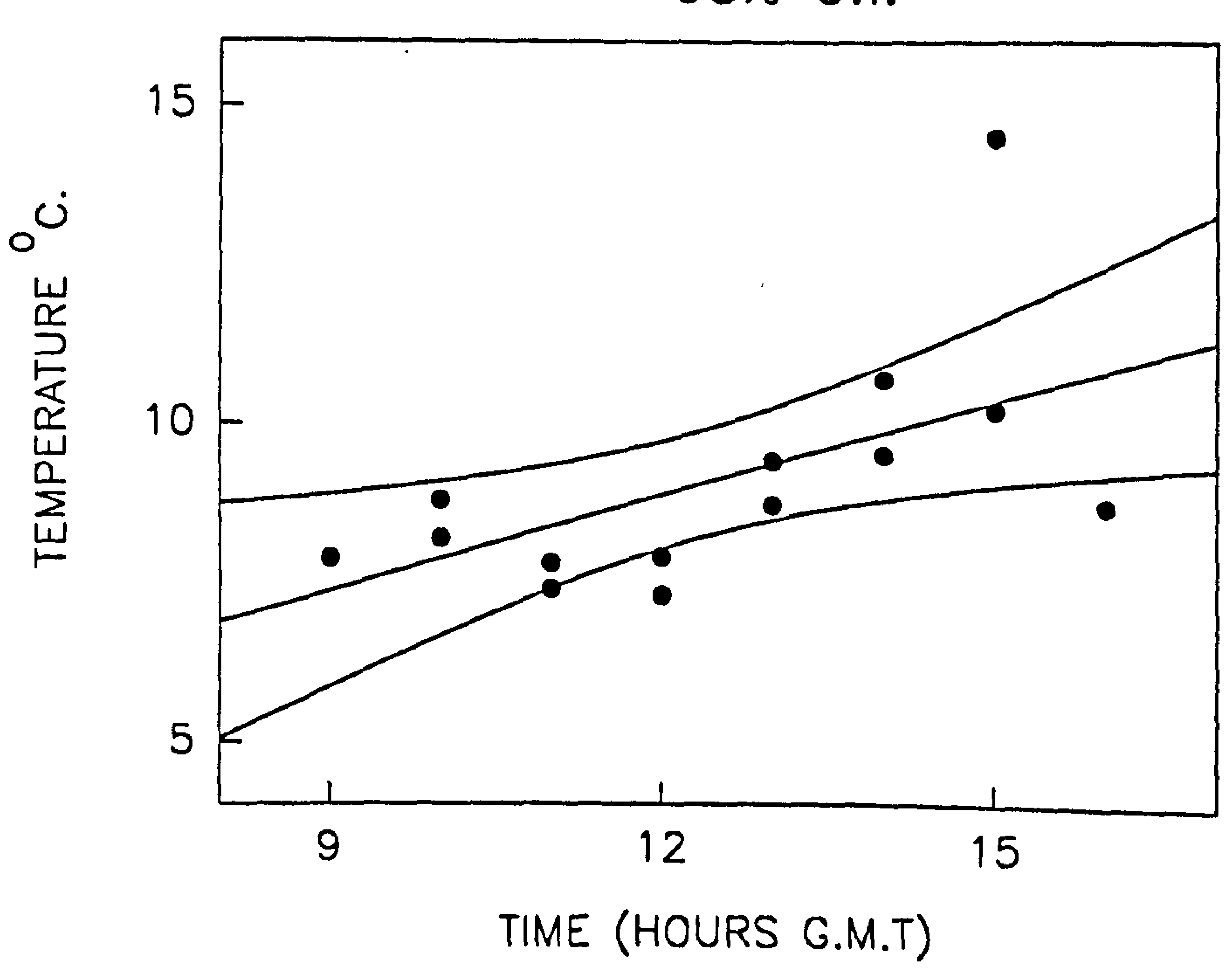


TABLE 34

SEASONALITY OF VARIABLES INVESTIGATED BY CORRELATION WITH TEMPERATURE

VARIABLE		S.U. 14-1	2	5	6	9	10-1	10-2	11-1	11-2	C-2	ALL SITES
pH INST	r	-	-	-	-	-	-	-	-	-	-	0.167
	n	-	-	-	-	-	-	-	-	-	-	382
pH COMP	r	-	-	-	-	-	-	-	-	-	-	0.193
	n	-	-	-	-	-	-	-	-	-	-	269
ABS.400	r	-	-	-	-	-	-	-	0.559	0.545	-	-
	n	-	-	-	-	-	-	-	22	26	-	-
ABS.250	r	0.607	-	-	-	-	-	-	0.593	0.632	-	0.228
	n	18	-	-	-	-	-	-	22	26	-	378
P.A.R.	r	-	0.835	0.854	-	0.852	-	-	-	-	0.838	0.628
	n	-	8	8	-	8	-	-	-	-	8	63
T.O.N.	r	-0.718	-	-0.723	-	-0.490	-0.506	-	-	-0.614	-	-0.341
	n	18	-	31	-	31	30	-	-	26	-	297
STONE CAROT.	r	0.944	-	-	-	-	-	-	-	-	-	-
	n	7	-	-	-	-	-	-	-	-	-	-

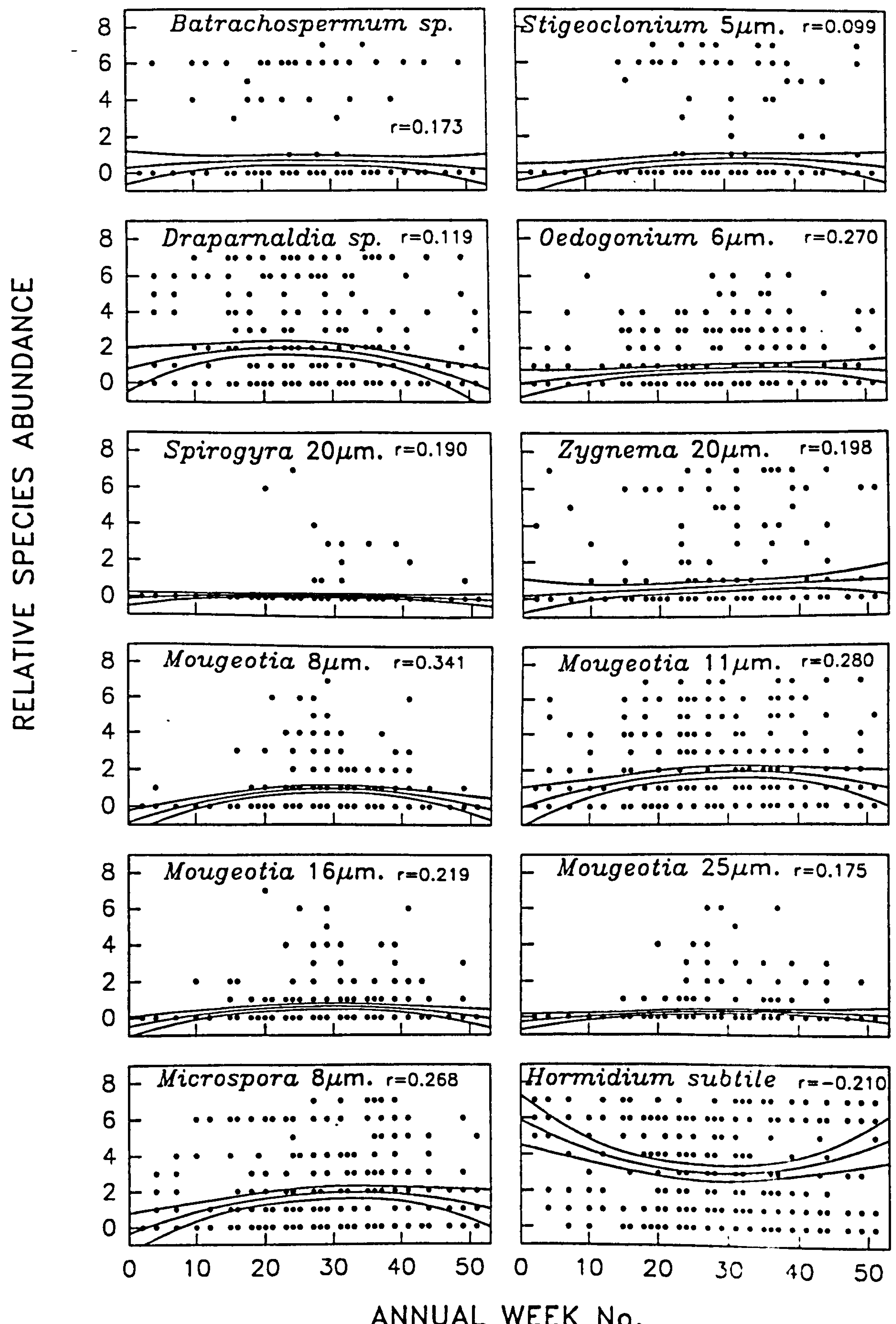
ONLY VALUES OF r SIGNIFICANT AT 1% ARE SHOWN

S.U.'s NOT REPRESENTED IN THE TABLE HAVE NOT BEEN TESTED

VARIABLES NOT REPRESENTED IN THE TABLE HAVE NO CORRELATIONS SIGNIFICANT AT 1%

FIG.26: PLOTS OF SPECIES ABUNDANCE AGAINST ANNUAL WEEK, WITH 2nd ORDER REGRESSIONS FITTED (99% C.I.)

r= CORRELATION WITH TEMPERATURE



4.4. SPECIES DIVERSITY

The diversity of species which compose a community has been considered an indicator of the degree of disturbance of an ecosystem; disturbance causing a reduction in the number and diversity of species (Stokes, 1986; Schindler, 1987; Rott and Pfister, 1988; Pontasch *et al* ,1989).

'Species diversity' is considered to consist of two components: richness and evenness (Ludwig and Reynolds, 1988). Species richness may be indicated by the total number of species in a sample, provided the sample size is uniform between sampling units (sites) (Ludwig and Reynolds, 1988). Indices which correct for different sample sizes have been proposed, but these are difficult to apply in a situation where an actual count is not taken, and overall the sample size is likely to even out when a large number of individual samples are combined to form the complete data matrix. Evenness expresses the degree to which the numbers of each species present are the same. Diversity indices attempt to combine the concepts of richness and evenness into a single index, which makes the relative contribution of these two factors difficult to assess (Ludwig and Reynolds, 1988).

4.4.1 Measures of Richness, Diversity and Evenness

The program SPDIVERS.BAS (Ludwig and Reynolds, 1988) computes several indices for diversity and evenness. The authors recommend Hill's numbers N1 and N2 as measures of diversity, and the value E5 (modified Hill's ratio) as a measure of evenness, as it is independent of sample size.

Species number, diversity and evenness for the data matrices A, B, C and D are shown in Table 35.

Evenness is quite high and consistent, the values lying between 0.6 and 0.9 in A, B and C and 0.8 and 1.0 in matrix D, implying that the communities are not excessively dominated by a few species (potential values of E5 range from 0 to 1.25). In fact this situation may stem from the sampling method and estimation of relative abundance, which has a similar effect to carrying out a log-transformation of true abundance data, reducing the range of abundance values in matrix C to 0-550 (while pooled p/a data range from 0 to 32), rather than over several logs.

The influence of environmental variables on richness, diversity and evenness has been investigated by correlation analysis of site-mean abundance data for matrices A, B and C, and using indices calculated on Matrix B pooled presence-absence data (Tables 36-39). Variables showing no correlations significant at least at the 5% level have been excluded from the tables. Correlations with richness and diversity are highly significant for pH, with higher correlations being obtained for pH from composite water samples. Similarly the correlations with H^+ ion are higher with composite samples, and significant correlations with A_{250} are found only with composite data. The composite sample values are more likely to represent true means (time-averaged) of these variables.

Strong correlations are obtained also with aluminium and calcium concentrations, but 'Flow', Mg^{2+} and silicate give variable results in different matrices and therefore these correlations must be considered less trustworthy. However if Matrix B is considered to contain the most complete data set, with all sites being sampled throughout its duration, then silicate is seen to give strong correlations with richness and diversity, significant at 1%. This is also seen in the 'unbiased' Matrix B pooled p/a data set (Table 39).

Evenness gives very low correlations with most variables, except in Matrix B (abundance data) where high correlations are found with 'Flow', aluminium and H^+.

A high correlation with richness or diversity implies an effect of the variable on the number of species composing the community. A lowered diversity is considered to indicate a stressed ecosystem. Therefore a decrease in pH (increased H^+), decrease in alkalinity, or increase in concentration of aluminium species may constitute environmental stress. However all these variables are strongly intercorrelated and thus it is not possible to determine whether they each have some unique influence on richness and diversity.

The strong negative correlations in Matrix B between forms of aluminium and evenness implies that the distribution of species abundances within the community is affected by Al; at higher Al concentrations the abundance of some species is increased while that of others is decreased.

As with all correlations, this cannot be taken as proof of a causative relationship since many of the environmental variables are highly correlated with each other. Furthermore the relationship between abundance of a species and a variable may not be straightforward; Lehman (1976) showed that a negative correlation between abundance of *Dinobryon* and phosphate concentration was not due to toxicity of phosphate to *Dinobryon*, as had been previously supposed, but was a result of its ability to scavenge very low concentrations of phosphate. Thus it effectively outcompetes other algae under these conditions, but is overgrown by faster growing algae under conditions of higher PO_4 concentration.

However, in view of the known toxic effects of Al on fish (Birchall *et al*, 1989; Dietrich and Schlatter, 1989; Grahn, 1980; Holtze and Hutchinson, 1989), invertebrates (Ormerod *et al*,, 1987; Weatherley *et al*, 1988), and algae (Claesson and Tornqvist, 1989; Folsom *et al*, 1986; Gensemer, 1990; Helliwell *et al* , 1983; Pillsbury and Kingston, 1990), it is at least possible that the observed correlations may indicate selective toxicity of Al towards some taxa.

4.4.2 Species Number in Each Sample as a Measure of Richness

Linear regression analysis has been carried out for species richness on the individual data points obtained for total species number at each site on each sampling occasion, giving a total number of data points ranging from 29 (Al-l) to 412 (pH). The regressions have been plotted along with the 99% CI (Figs. 27a, b). The p-values of the regression and correlation coefficient 'r' are shown for pH and A_{350}. Many of the confidence intervals are fairly narrow indicating a high degree of reliability. Steep slopes indicating a strong effect of the variable on species number are obtained for pH, H^+ (not shown), temperature, (labile) Aluminium, akalinity, Ca^{2+}, Mg^{2+} and SiO_2. Other variables either have a shallow slope or a wide 99% C.I., which means that the slope is not significantly different from zero.

These correlated variables are all acidification-related, so that there is clearly a decrease of richness and diversity with increasing acidity. It is less clear whether this decrease can be related to ecosystem disturbance (Schindler 1987), since the conditions in each burn have fluctuated naturally about the mean over several seasons. For the premise of low species richness indicating a disturbed ecosystem to hold true, the intra-seasonal range of e.g. pH, would have to be greater in low-mean pH burns.

TABLE 35
SPECIES RICHNESS, DIVERSITY AND EVENNESS

	14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	W
MATRIX A															
SP. NO.	-	8	10	12	10	15	3	8	9	8	11	10	17	22	11
DIV. N1	-	7.6	17.5	10.4	12.3	16.3	3.0	7.8	10.2	8.6	10.8	8.6	16.2	23.9	8.4
N2	-	5.7	13.0	7.4	9.9	13.0	2.7	6.0	8.0	7.1	9.6	6.7	10.6	20.1	7.0
EV. E5	-	.72	.73	.68	.79	.79	.82	.73	.76	.81	.88	.75	.63	.83	.82
MATRIX B															
SP. NO.	9	9	14	16	14	21	12	7	7	8	7	9	11	23	18
DIV. N1	9.3	8.2	14.4	16.7	13.6	20.3	14.5	5.6	6.8	5.6	7.2	6.6	14.8	22.6	22.2
N2	6.9	6.8	10.1	13.9	11.0	15.4	11.4	4.0	4.6	3.6	5.1	4.9	11.7	17.0	15.8
EV. E5	.71	.81	.68	.82	.79	.75	.77	.66	.62	.57	.66	.70	.78	.74	.70
MATRIX C															
SP. NO.	9	9	14	16	14	21	12	8	9	8	11	10	17	23	18
DIV. N1	9.3	8.3	17.7	15.6	14.1	20.0	14.7	6.8	9.4	7.1	10.2	8.0	18.7	27.2	22.1
N2	6.9	6.5	12.7	12.1	11.1	15.0	11.4	4.9	6.5	4.7	8.3	5.9	12.7	21.4	15.7
EV. E5	.71	.75	.70	.77	.77	.74	.76	.67	.65	.60	.79	.71	.66	.78	.69
MATRIX D															
SP. NO.	-	6	10	7	7	15	-	10	-	3	-	6	-	17	-
DIV. N1	-	4.9	8.9	6.5	6.4	13.0	-	7.5	-	3.0	-	4.9	-	14.5	-
N2	-	4.6	8.2	6.2	6.1	12.1	-	6.3	-	3.0	-	4.3	-	13.4	-
EV. E5	-	.91	.91	.94	.95	.92	-	.82	-	1.02	-	.85	-	.92	-
MATRIX B (Pooled P/A)															
SP. NO.	9	9	14	16	14	21	12	7	7	8	7	9	11	23	18
DIV. N1	12.9	10.0	20.7	19.7	17.1	27.0	17.4	8.9	10.3	9.0	10.8	10.3	18.2	28.7	29.3
N2	10.9	10.2	18.4	18.4	15.4	25.2	16.9	7.7	10.1	7.2	10.8	8.9	17.3	27.6	27.9
EV. E5	.84	1.02	.88	.93	.90	.93	.97	.85	.97	.77	1.01	.86	.95	.96	.95

TABLE 36

CORRELATIONS BETWEEN MEASURES OF SPECIES RICHNESS, DIVERSITY AND EVENNESS FOR EACH SITE WITH MEAN VALUES OF ENVIRONMENTAL VARIABLES; MATRIX A, abundance

	RICHNESS: SPECIES NO.	DIVERSITY N1	DIVERSITY N2	EVENNESS E5
pH: instantaneous	0.675*	0.618	0.559	-0.106
pH: composite	0.870**	0.894**	0.852**	0.156
Flow	0.770**	0.552	0.529	-0.221
Al. total monomeric	-0.676*	-0.638	-0.581	0.092
Al. non-labile	-0.668*	-0.632*	-0.559	0.221
Al. labile	-0.643*	-0.606	-0.561	0.014
Alkalinity	0.946**	0.944**	0.934**	0.295
Ca^{2+}	0.859**	0.883**	0.885**	0.338
Mg^{2+}	0.739*	0.759*	0.779**	0.390
SiO_2	0.180	0.271	0.348	0.606
A_{250} composite	-0.285	-0.362	-0.297	0.225
H^+ instantaneous	-0.596*	-0.624	-0.552	0.178
H^+ composite	-0.654*	-0.688*	-0.628*	0.057
DIVERSITY N1	0.890**			
DIVERSITY N2	0.872**	0.980**		
EVENNESS E5	-0.097	-0.066	0.121	

SIGNIFICANCE LEVEL *5% **1%

TABLE 37

CORRELATIONS BETWEEN MEASURES OF SPECIES RICHNESS, DIVERSITY AND EVENNESS FOR EACH SITE WITH MEAN VALUES OF ENVIRONMENTAL VARIABLES; MATRIX B, abundance

	RICHNESS: SPECIES NO.	DIVERSITY N1	DIVERSITY N2	EVENNESS E5
pH: instantaneous	0.797**	0.907*	0.877**	0.376
pH: composite	0.950**	0.954**	0.935**	0.526
Flow	0.683	0.571	0.652	0.750**
Al. total monomeric	-0.760	-0.791**	-0.837**	-0.773**
Al. non-labile	-0.709*8	-0.699**	-0.747**	-0.688**
Al. labile	-0.765**	-0.803**	-0.842**	-0.790**
Alkalinity	0.811**	0.794**	0.755**	0.278
Ca^{2+}	0.690*	0.678*	0.637*	0.236
Mg^{2+}	0.569	0.539	0.480	-0.037
SiO_2	0.923**	0.896**	0.871**	0.150
A_{250} composite	-0.571	-0.609*	-0.630*	-0.497
H^+ instantaneous	-0.771**	-0.867**	-0.883**	-0.621*
H^+ composite	-0.815**	-0.850**	-0.875**	-0.756**
DIVERSITY N1	0.950**			
DIVERSITY N2	0.942**	0.991**		

SIGNIFICANCE LEVEL *5% **1%

TABLE 38

CORRELATIONS BETWEEN MEASURES OF SPECIES RICHNESS, DIVERSITY AND EVENNESS FOR EACH SITE WITH MEAN VALUES OF ENVIRONMENTAL VARIABLES; MATRIX C, abundance

	RICHNESS: SPECIES NO.	DIVERSITY N1	DIVERSITY N2	EVENNESS E5
pH: instantaneous	0.819**	0.912**	0.871**	0.152
pH: composite	0.885**	0.934**	0.908**	0.302
Flow	0.638*	0.441	0.512	0.575
Al. total monomeric	-0.779**	-0.769**	-0.783**	-0.555*
Al. non-labile	-0.672**	-0.670**	-0.692**	-0.479
Al. labile	-0.775**	-0.757**	-0.777**	-0.633*
Alkalinity	0.815**	0.899**	0.899**	0.418
Ca^{2+}	0.669*	0.788**	0.790**	0.332
Mg^{2+}	0.540	0.668*	0.660*	0.094
SiO_2	-0.008	0.129	0.137	0.110
A_{250} composite	-0.448	-0.461	-0.432	-0.061
H^+ instantaneous	-0.752**	-0.813**	-0.800**	-0.342
H^+ composite	-0.765**	-0.770**	-0.769**	-0.542
DIVERSITY N1	0.960**			
DIVERSITY N2	0.961**	0.991**		
EVENNESS E5	0.390	0.332	0.438	

SIGNIFICANCE LEVEL *5% **1%

TABLE 39

CORRELATIONS BETWEEN MEASURES OF SPECIES RICHNESS, DIVERSITY AND EVENNESS FOR EACH SITE WITH MEAN VALUES OF ENVIRONMENTAL VARIABLES; MATRIX B, P/A

	RICHNESS: SPECIES NO.	DIVERSITY N1	DIVERSITY N2	EVENNESS E5
pH: instantaneous	0.797**	0.911**	0.901**	0.192
pH: composite	0.950**	0.953**	0.949**	0.230
Flow	0.683	0.501	0.492	0.150
Al. total monomeric	-0.760**	-0.741**	-0.735**	-0.274
Al. non labile	-0.709**	-0.648**	-0.640**	-0.212
Al. labile	-0.765**	-0.761**	0.756**	-0.303
Alkalinity	0.811**	0.806**	0.819**	0.239
Ca^{2+}	0.690*	0.684	0.701*	0.237
Mg^{2+}	0.569	0.567	0.586	0.155
SiO_2	0.923**	0.924**	0.921**	0.189
A_{250} composite	-0.571	-0.560	-0.548	-0.110
H^+ instantaneous	-0.771**	-0.825**	-0.814**	-0.225
H^+ composite	-0.815**	-0.801**	-0.794**	-0.259
DIVERSITY N1	0.951**			
DIVERSITY N2	0.943**	0.995**		
EVENNESS E5	0.194	0.254	0.344	

SIGNIFICANCE LEVEL *5% **1%

FIG. 27a :- PLOTS, REGRESSIONS AND 99% C.I. OF SPECIES No. IN EACH SAMPLE WITH ENVIRONMENTAL VARIABLES

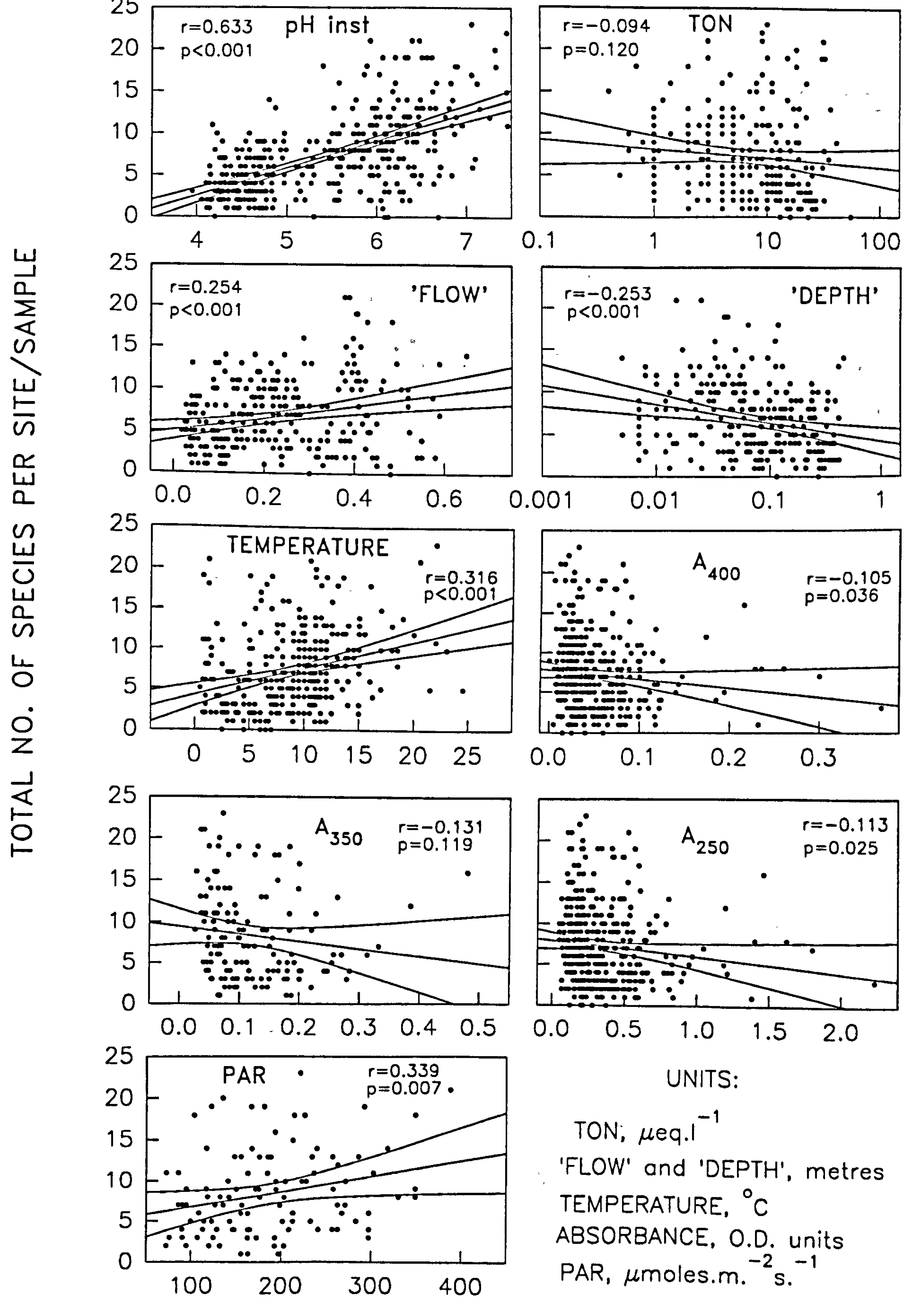
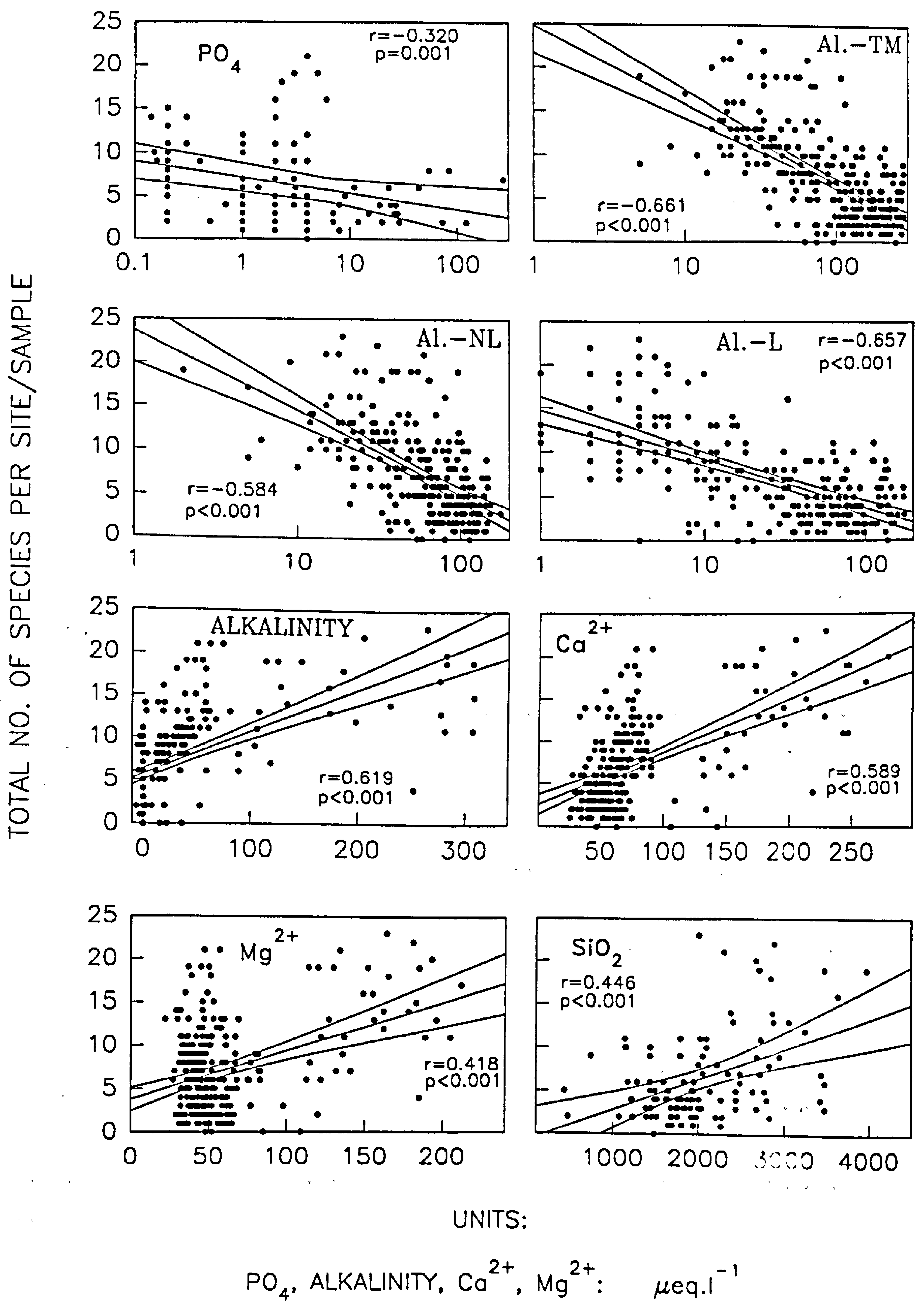


FIG.27b:– PLOTS, REGRESSIONS AND 99% C.I. OF SPECIES No. IN EACH SAMPLE WITH ENVIRONMENTAL VARIABLES



UNITS:

PO_4, ALKALINITY, Ca^{2+}, Mg^{2+}: $\mu eq.l^{-1}$

Al, SiO_2 : $\mu g.l^{-1}$

The significance of temperature may be spurious, since a plot of mean temperature against time of sampling for each site yields a convincing linear relationship (Fig. 25b). The sampling sites were visited in approximately the same order and therefore at about the same time of day, on each sampling trip. It appears therefore that the differences found between sites are largely a function of the total daily insolation up to the time of measurement (Wong *et al*, 1978) and hence a simple measurement of field temperature is inadequate to properly characterize a site. Integrating temperature recorders similar to those used for PAR (Woodward and Yaqub, 1979; Mitchell and Woodward, 1987) would be necessary.

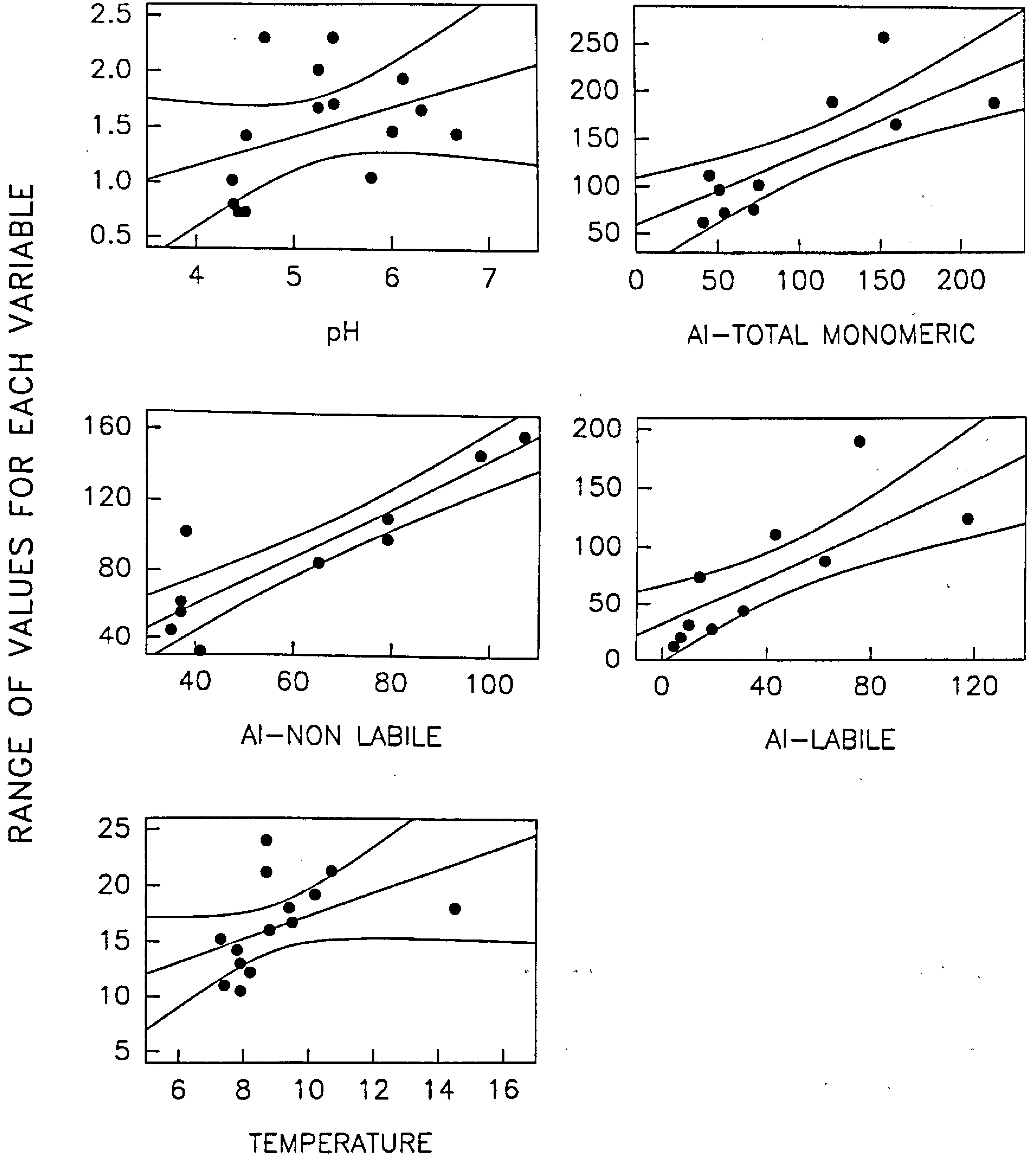
A limited number of continuous traces of temperature have been recorded by SOAFD, and these show changes of 6°C in 8 hours are possible in summer (unpublished observations).

Plots of regression of range (maximum-minimum) on mean for pH, and forms of Al (Fig. 28) have a positive slope which is significant at 5% for Al but not for pH. It is therefore possible that the large excursions in aluminium concentration found at the upper range of mean [Al], and hence in the more acidic streams, do constitute a major ecosystem disturbance.

Episodes of high [Al] in streams have been shown to result in fish kills (Grahn, Ormerod *et al*, 1987; Dietrich and Schlatter, 1989; Holtze and Hutchinson, 1989) increased drift of benthic invertebrates (Ormerod *et al*, 1987; Weatherley *et al*, 1988) and may effect changes in succession of phytoplankton (Pillsbury and Kingston, 1990). Differential sensitivity of algae to Al has been shown (Claesson and Tornqvist, 1989) and it is thus possible that pulses of Al occurring in acid episodes may also influence benthic algae, stressing more sensitive species and possibly eliminating them from the community.

In addition Burn 9 characteristically has a high A_{250}, due to the presence of humic acids. Both PO_4^{3-} and organic acids may reduce the toxicity of Al by complexation. Thus the differences found in growth experiments between the high and low Al treatments and B9 medium may reflect differences found in the field between circumneutral (low-Al), acidified humic and acidified non-humic streams.

FIG.28:- PLOTS AND REGRESSIONS (+95% C.I.) OF MEANS ON RANGE FOR SELECTED ENVIRONMENTAL VARIABLES



SITE-AVERAGE VALUES OF VARIABLES

4.5 GROUPING OF SPECIES BY NASSOC.BAS (INVERSE ASSOCIATION ANALYSIS)

This program is designed to group Sampling Units by similarity of species composition (as described in Section 4.5.3), but if used with an inverted data matrix can equally be used to group species by their presence of absence in SUs, allowing a dendrogram to be constructed.

Sampling sites in this study correspond to Sampling Units (SUs) in the terminology used in statistical ecology (Ludwig and Reynolds, 1988). The term SU has been used when referring to the analyses carried out by the Statistical Ecology programs (Ludwig and Reynolds, 1988), but 'sites' are referred to as such throughout the rest of the text.

Dendrograms have been drawn of the associations between species in matrices A, B and C, using both χ^2 and Yate's corrected χ^2 as the basis for division. Division has been continued as far as the program will permit or until homology is achieved (Figs. 29-31).

As with Normal Association Analysis the corrected χ^2 gives a less divisive dendrogram, with fewer final groups. At the first division, both χ^2 and corrected χ^2 split the species identically. Matrix A groups some species differently from B and C. Three broad species associations may be distinguished (Table 40). The first includes those species always present in the upper grouping in the diagrams, split off at the first division. These are species more commonly found in acidified sites. The second species group comprises species always occurring in the lower first-division grouping, and contains species most common in the least acidified sites. The third group contains species which may sometimes be found in the first or the second groups. In addition it must be pointed out that some of the species occurring in the lowest final group in the diagrams are there essentially by default, being present in only one or two sites (or not at all in Matrix A). The third, indeterminate group may comprise truly ambiguous species, or species which are sensitive to some change which has occurred between the earlier and later parts of the sampling programme.

Subdivision of the original data set into smaller sets more spread out in time and paired with regard to season, was carried out in order to determine whether this latter possibility is the case. Subsets using species data from one to three sampling dates were made up in matched pairs. Not all sites are represented in most of these subsets (Table 41). The following discussion relies mainly on the analyses using the corrected χ^2.

Subsets 1 and 2 (Figs. 32, 33) compare midsummer samples taken in 1986 and 1985. In 1986 the first division splits off the 'acidobiontic' group of species containing *Scytonema* sp and *Hormidium* 8 µm. which do not occur in 1987 (Subset 2). In 1987 however this group also includes *Oedogonium* 6 µm., *Zygnema* sp., *Mougeotia* 5 µm, and *Geminella* 11µm.

FIG.29

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

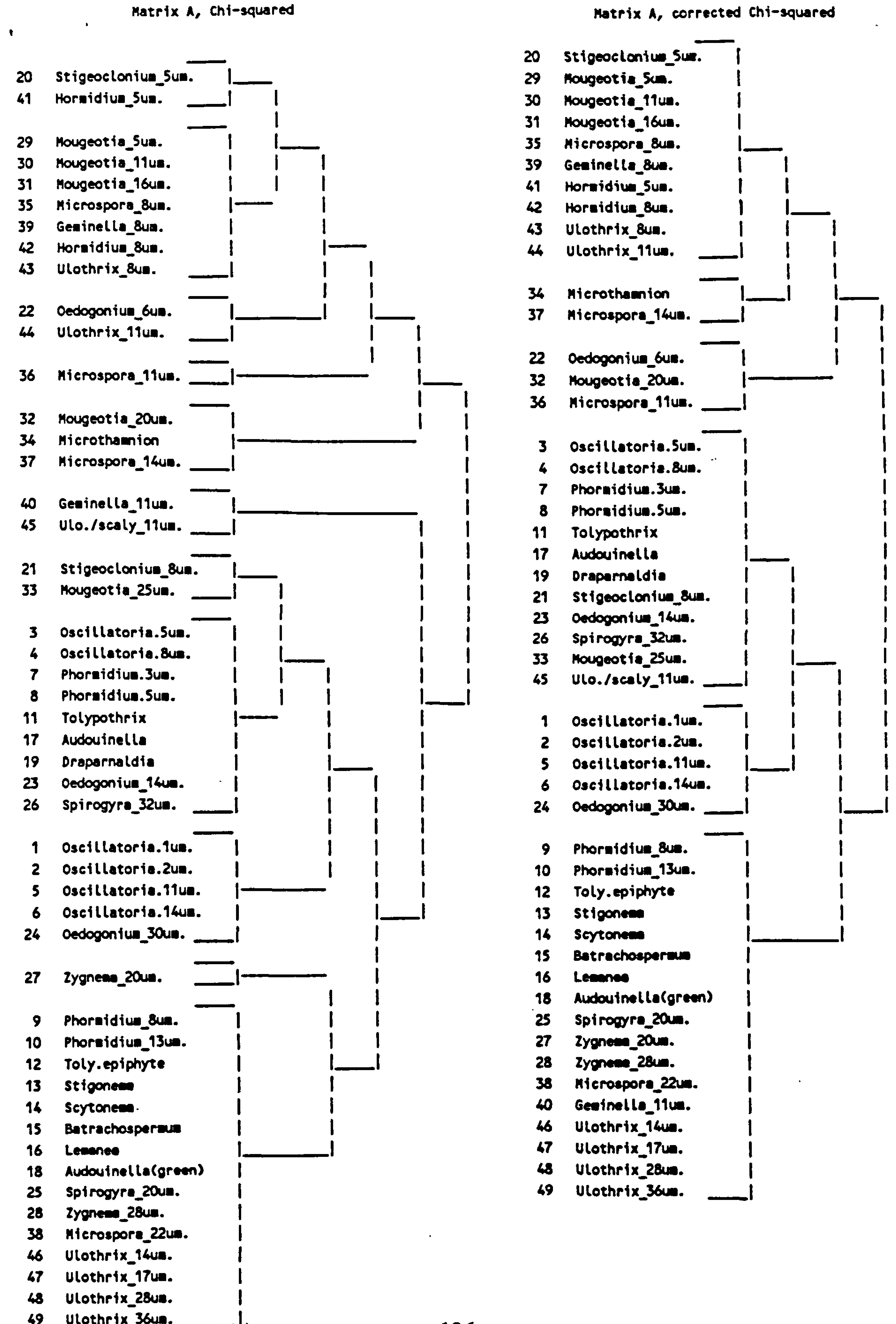


FIG.30

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

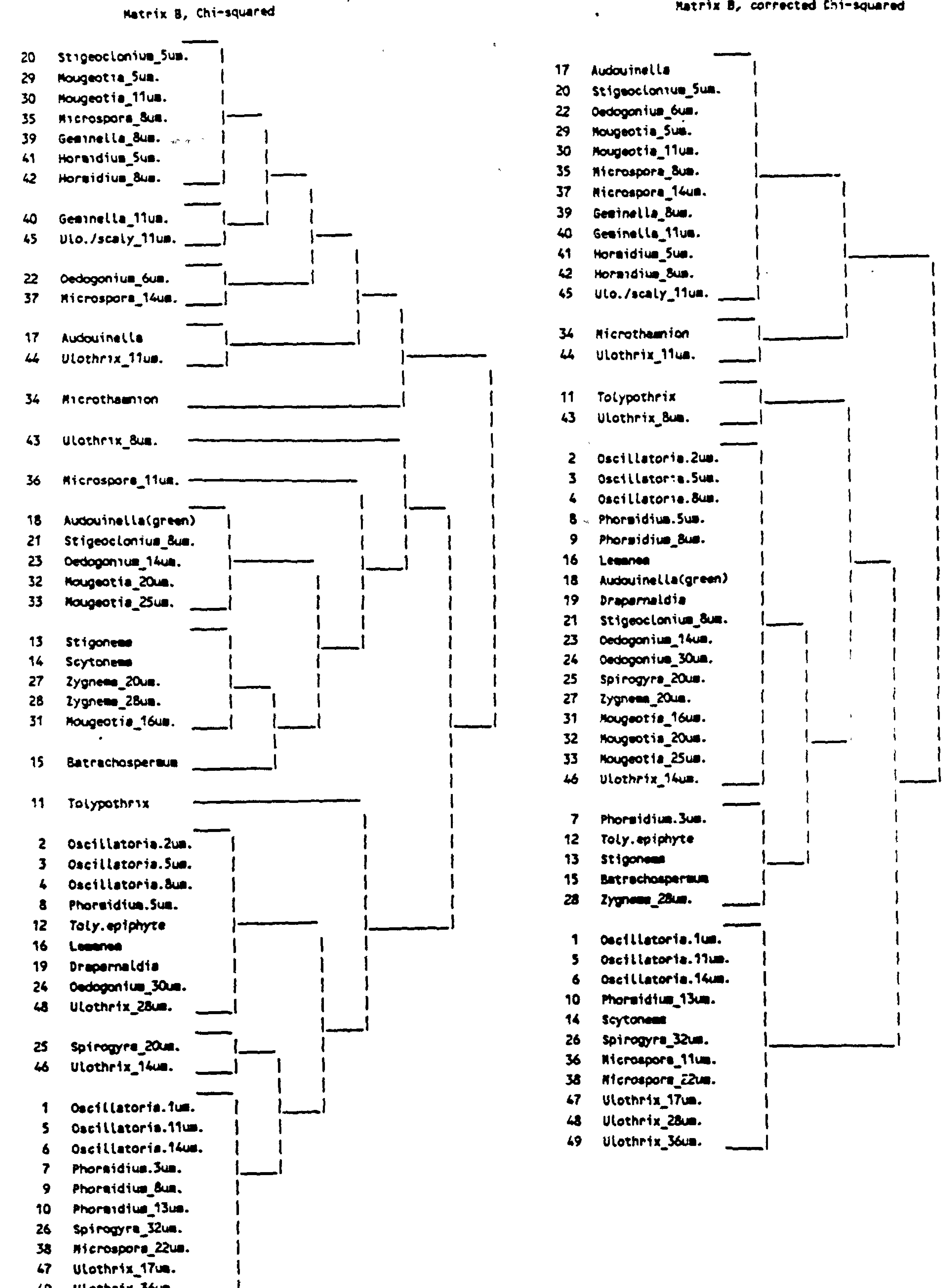


FIG.31

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

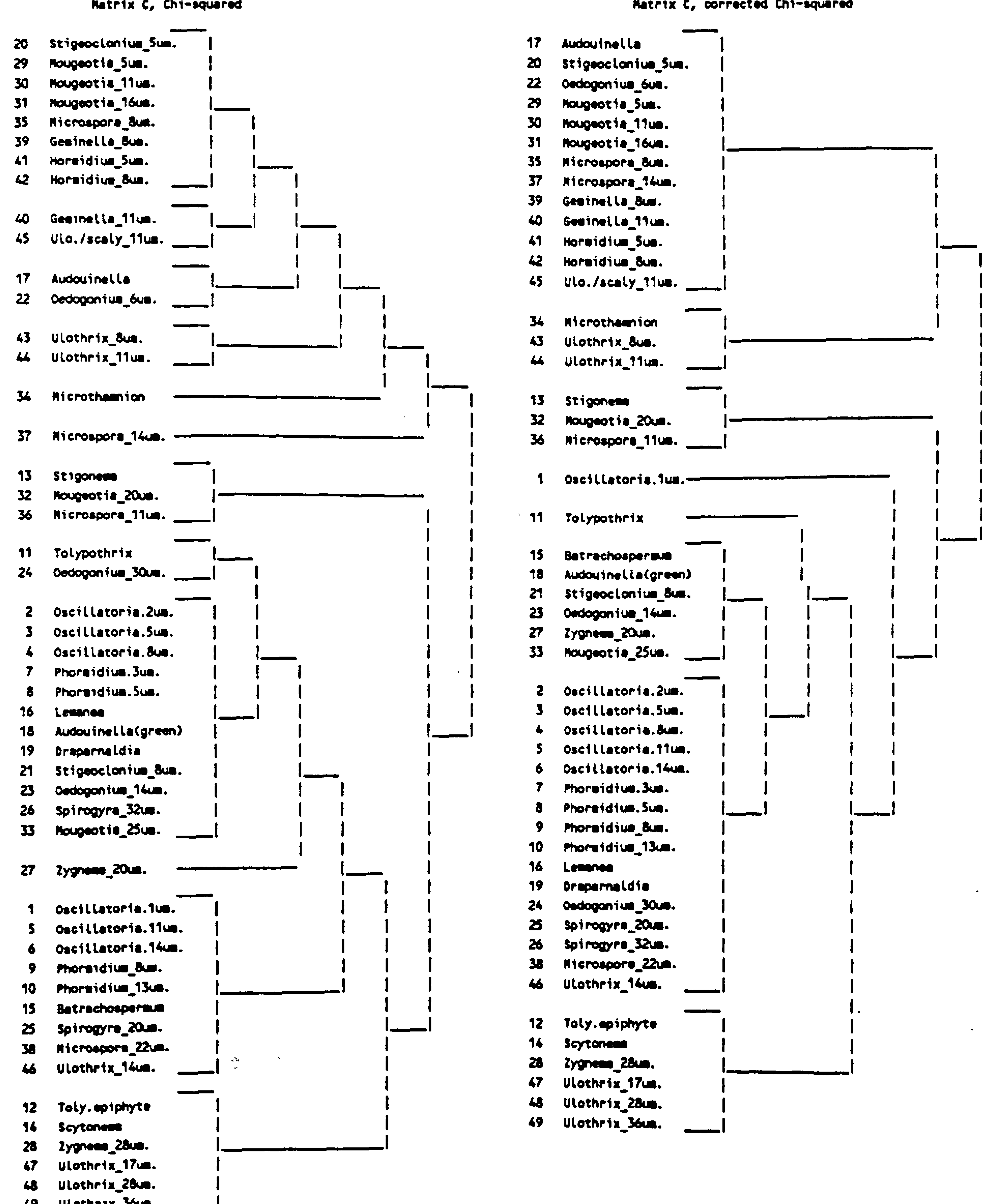


TABLE 40
SPECIES GROUPS REVEALED BY INVERSE
ASSOCIATION ANALYSIS ON MATRICES A, B, AND C

1. 'Acidobiontic'		2. Remnant		3. Indeterminate
Stigeoclonium 5 µm.		*Oscillatoria* spp.		*Audouinella*
Oedogonium 6 µm.	*	*Phormidium* spp.		*Mougeotia* 20 µm.
Mougeotia 5 µm.	*	*Tolypothrix*		*Geminella* 11 µm.
M. 11 µm.		Epiphyte of *Tolypothrix*		*Ulothrix* -sc. 11 µm.
M. 16 µm.	*	*Stigonema* sp.		
Microthamnion	*	*Scytonema* sp.	*	
Microspora 8 µm.		*Lemanea* sp.		
M. 14 µm.	*	*Batrachospermum* sp.		
Geminella 8 µm.	*	*Audouinella* (green)		
Hormidium 5 µm.		*Stigeoclonium* 8 µm.		
H. 8 µm.	*	*Draparnaldia* sp.		
Ulothrix 8 µm.	*	*Oedogonium* 14 µm.		
U. 11 µm.	*	*O.* 30 µm.		
		Spirogyra spp.		
		Zygnema spp.		
		Mougeotia 25 µm.		
		Microspora 11 µm.	*	
		M. 22 µm.		
		Ulothrix 14 µm.		
		U. 17 µm.		
		U. 28 µm.		
		U. 36 µm.		

*These species would be classified indeterminate taking the results of subset analyses into account

Subsets 3 and 4 (Figs. 34, 35) compare midsummer 1986 with 1988. The 'acidobiontic' group includes *Oedogonium* 6 µm. in Subset 3 (contrasting with its absence from Subset 1, also ostensibly representing midsummer 1986), and *Microthamnion* sp., but omits *Mougeotia* 5µm., *Microspora* 14 µm., *Geminella* 8 µm., *G.* 11 µm., and *Ulothrix* 11µm., all of which are present in Subset 4.

Subsets 5 and 6 (Figs. 36, 37) compare 1987 with 1988. Comparing first Subsets 5 and 2 (both from 1987), essentially the same grouping of 'acidobiontic' species is found with the exception of *Zygnema* 20 µm., which however is split separately from the remnant in Subset 5. These two subsets include samples from the same dates, but Subset 5 includes all S.U.s in the analysis while 2 omits 14-1, 5/6 and Ward. Thus the results of the analysis are sensitive to the amount of information included.

Subset 6 omits *Oedogonium* 6 µm. but includes *Hormidium* 8 µm. and *Ulothrix* 11µm, and *Mougeotia* 16 µm is replaced by *Mougeotia* 20 µm. Comparing Subset 6 with 4, *Mougeotia* 20 µm. is missing from the 'acidobiontic' group in the latter. Subset 4 omits five SUs.

Subsets 7 and 8 (Figs. 38, 39) and 10 and 11 (Figs. 40, 41) compare data from only one sample date. It is apparent that in Subset 7, 'acidobiontic' species are being split off in a piecemeal manner from the remnant group. Thus under certain conditions the division by NASSOC must be considered unstable, even using Yates corrected χ^2. Subset 8 gives very similar results to Subsets 4 and 6.

Subsets 10 and 11 differ from the other pairs in comparing two samples taken early and late in the same season, and including data from all 15 Sampling Units. Analysis by χ^2 of Subset 10 splits off at the first division six of the 'acidobiontic' species, but groups four others occurring in this group in other analyses, with the remnant, namely *Oedogonium* 6 µm., *Mougeotia* 16 µm. *M.* 20 µm. and *Geminella* 11 µm.

Using the corrected χ^2, these species are grouped together with *Stigonema* sp., *Batrachospermum* sp. and *Zygnema* 20 µm.

In Subset 11 with χ^2, the 'acidobiontic' species are broken up in several small groups, similar to the situation with Subset 7. Using the corrected χ^2 the first division splits off only three species, but the second includes most of the other 'acidobiontic' species, along with some others.

TABLE 41

INVERSE ASSOCIATION ANALYSIS BY NASSOC ON SUBSET PAIRS: DATES AND SAMPLING UNITS REPRESENTED IN EACH SET

Set no.	Dates	Set no.	Dates	S.U.s represented in data
1	28.5.86	2	9.6.87	Omit 14-1, 5/6, WARD
	19.8.86		7.7.87	
3	14.5.86	4	17.5.88	Omit 14-1, 5/6, 10-2, 11-1, WARD
	11.6.86		14.6.88	
	19.8.86		30.8.88	
5	9.6.87	6	14.6.88	All S.U.s represented
	7.7.87		3.8.88	
7	28.5.86	8	17.5.88	Omit 14-1, WARD
10	12.4.88	11	30.8.88	All S.U.s represented

FIG.32

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

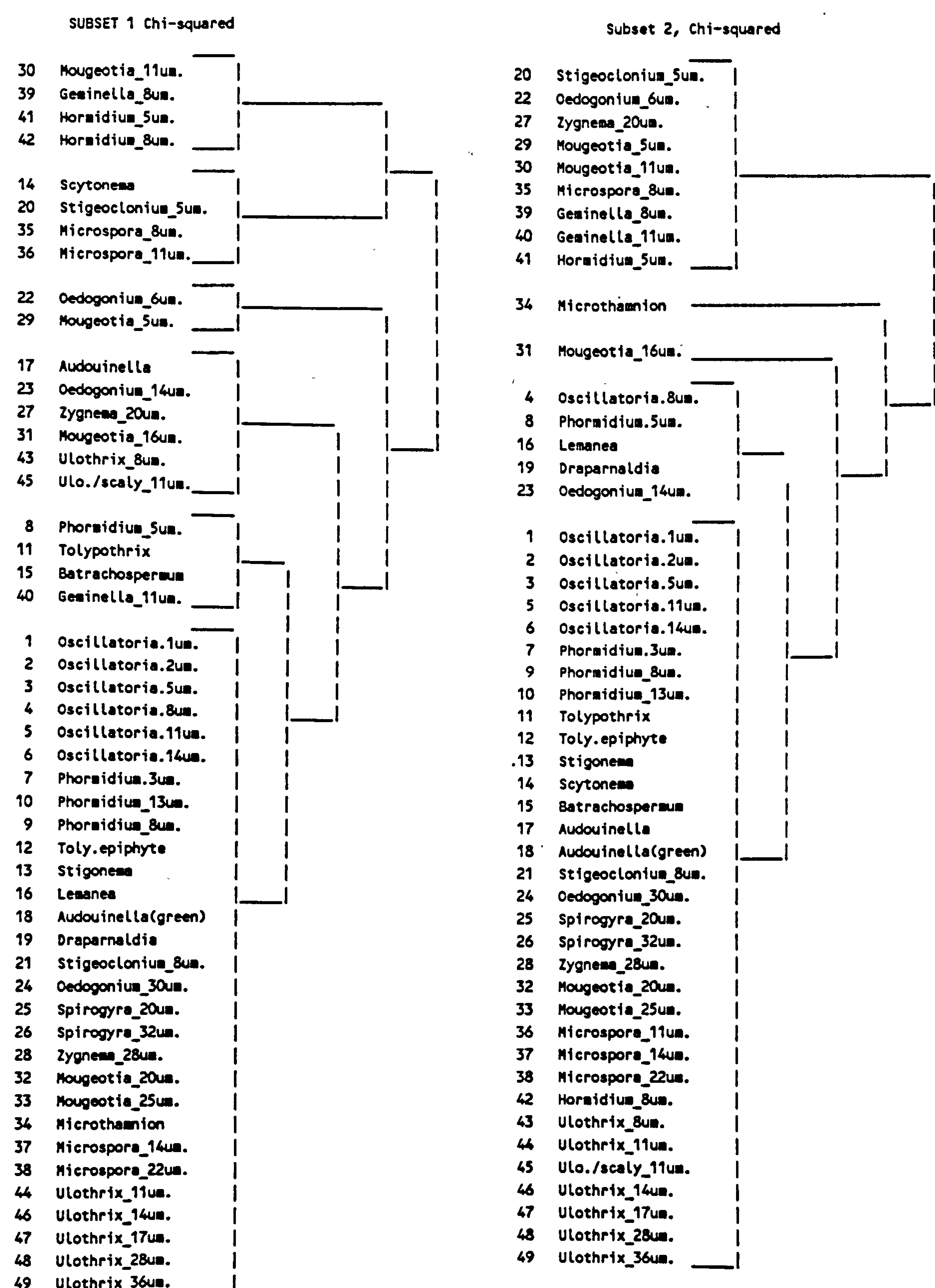


FIG.33

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

Subset 1, corrected Chi-squared

30 Mougeotia_11um.
39 Geminella_8um.
41 Hormidium_5um.
42 Hormidium_8um.

14 Scytonema
20 Stigeoclonium_5um.
35 Microspora_8um.
36 Microspora_11um.

29 Mougeotia_5um.

22 Oedogonium_6um.

1 Oscillatoria.1um.
2 Oscillatoria.2um.
3 Oscillatoria.5um.
4 Oscillatoria.8um.
5 Oscillatoria.11um.
6 Oscillatoria.14um.
7 Phormidium.3um.
8 Phormidium.5um.
9 Phormidium_8um.
10 Phormidium_13um.
11 Tolypothrix
12 Toly.epiphyte
13 Stigonema
15 Batrachospermum
16 Lemanea
17 Audouinella
18 Audouinella(green)
19 Draparnaldia
21 Stigeoclonium_8um.
23 Oedogonium_14um.
24 Oedogonium_30um.
25 Spirogyra_20um.
26 Spirogyra_32um.
27 Zygnema_20um.
28 Zygnema_28um.
31 Mougeotia_16um.
32 Mougeotia_20um.
33 Mougeotia_25um.
34 Microthamnion
37 Microspora_14um.
38 Microspora_22um.
40 Geminella_11um.
43 Ulothrix_8um.
44 Ulothrix_11um.
45 Ulo./scaly_11um.
46 Ulothrix_14um.
47 Ulothrix_17um.
48 Ulothrix_28um.
49 Ulothrix_36um.

Subset 2, corrected Chi-squared

20 Stigeoclonium_5um.
22 Oedogonium_6um.
27 Zygnema_20um.
29 Mougeotia_5um.
30 Mougeotia_11um.
35 Microspora_8um.
39 Geminella_8um.
40 Geminella_11um.
41 Hormidium_5um.

34 Microthamnion

4 Oscillatoria.8um.
8 Phormidium.5um.
16 Lemanea
19 Draparnaldia
23 Oedogonium_14um.

31 Mougeotia_16um.
1 Oscillatoria.1um.
2 Oscillatoria.2um.
3 Oscillatoria.5um.
5 Oscillatoria.11um.
6 Oscillatoria.14um.
7 Phormidium.3um.
9 Phormidium_8um.
10 Phormidium_13um.
11 Tolypothrix
12 Toly.epiphyte
13 Stigonema
14 Scytonema
15 Batrachospermum
17 Audouinella
18 Audouinella(green)
21 Stigeoclonium_8um.
24 Oedogonium_30um.
25 Spirogyra_20um.
26 Spirogyra_32um.
28 Zygnema_28um.
32 Mougeotia_20um.
33 Mougeotia_25um.
36 Microspora_11um.
37 Microspora_14um.
38 Microspora_22um.
42 Hormidium_8um.
43 Ulothrix_8um.
44 Ulothrix_11um.
45 Ulo./scaly_11um.
46 Ulothrix_14um.
47 Ulothrix_17um.
48 Ulothrix_28um.
49 Ulothrix_36um.

FIG.34

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

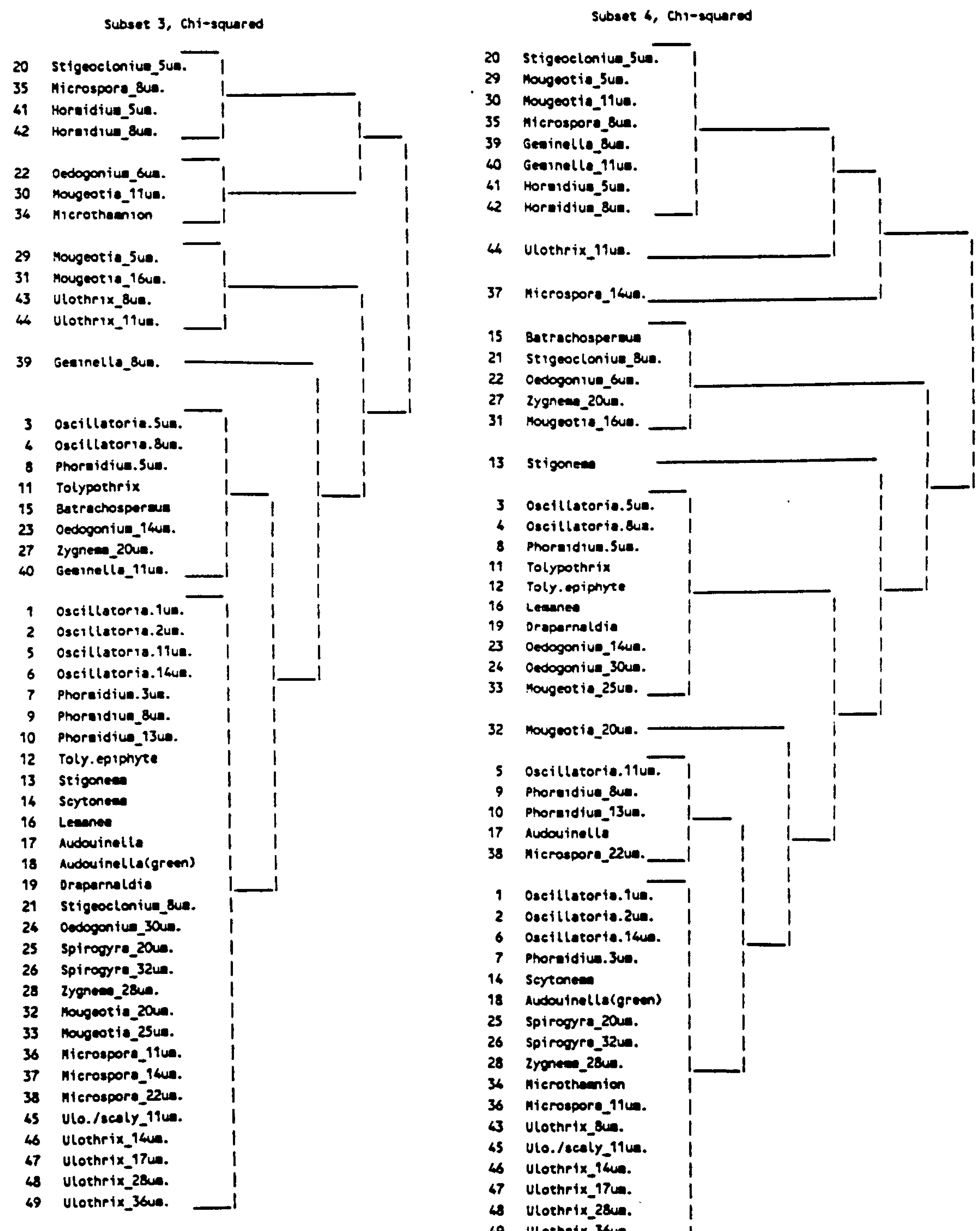


FIG.35

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

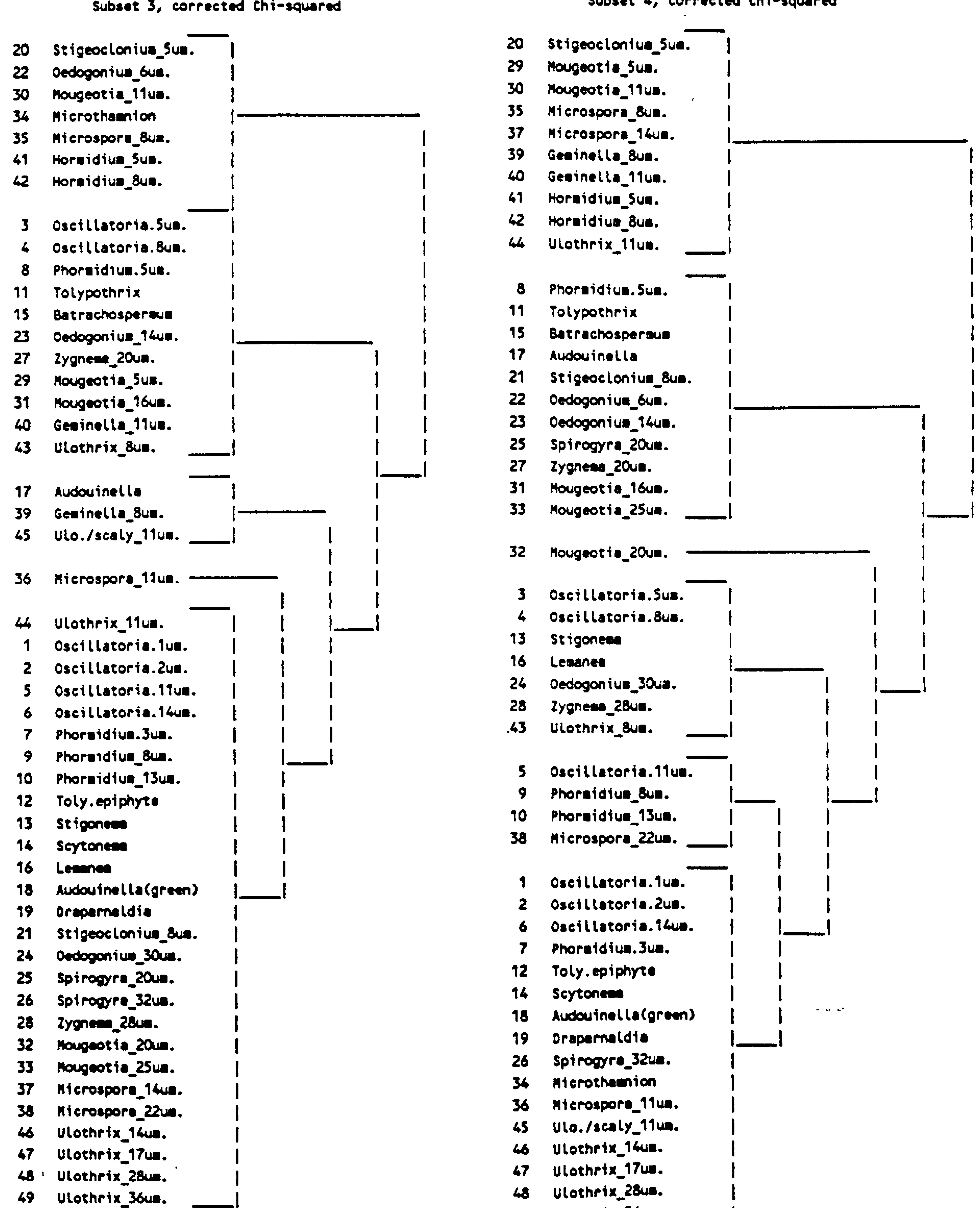


FIG.36

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

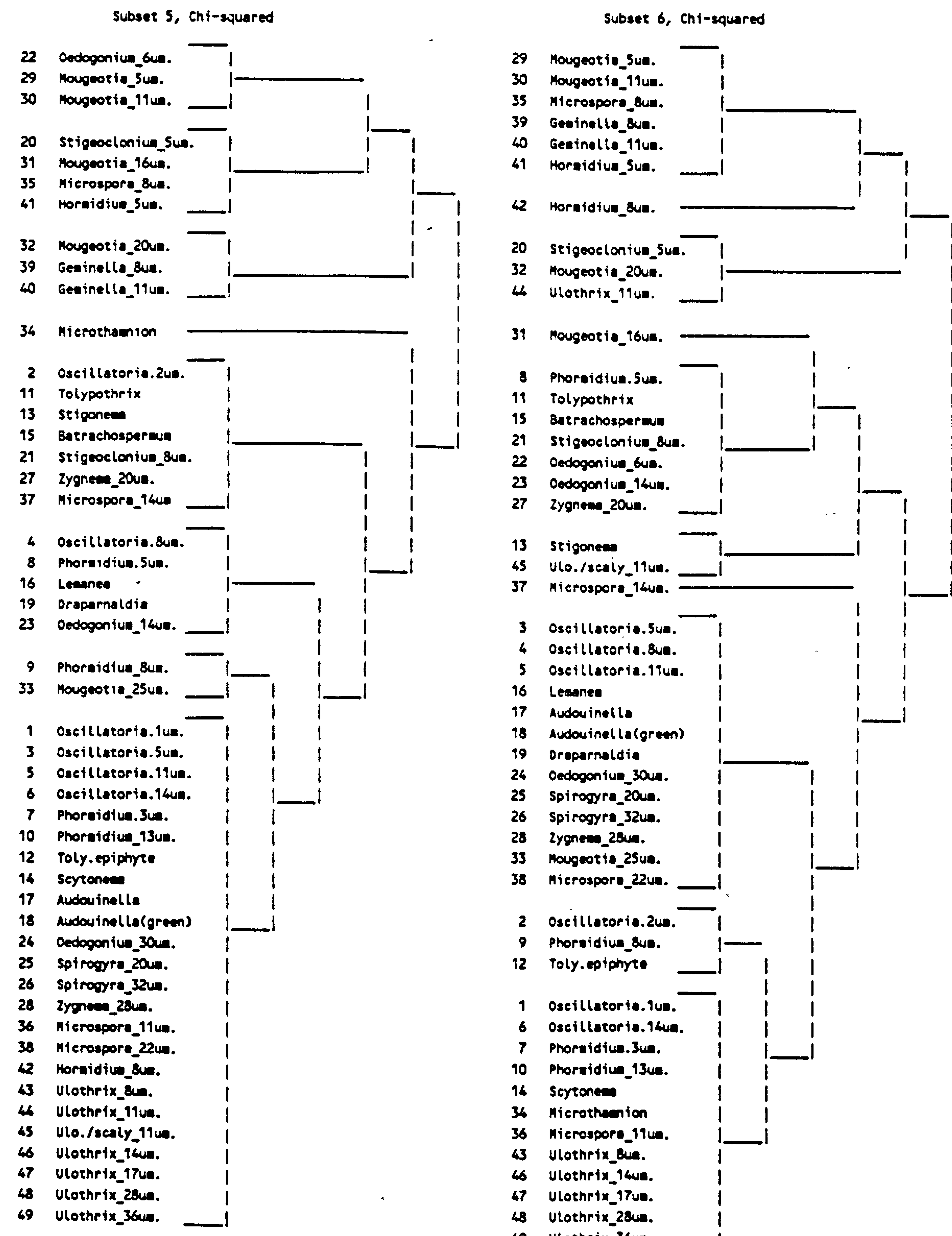


FIG.37

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

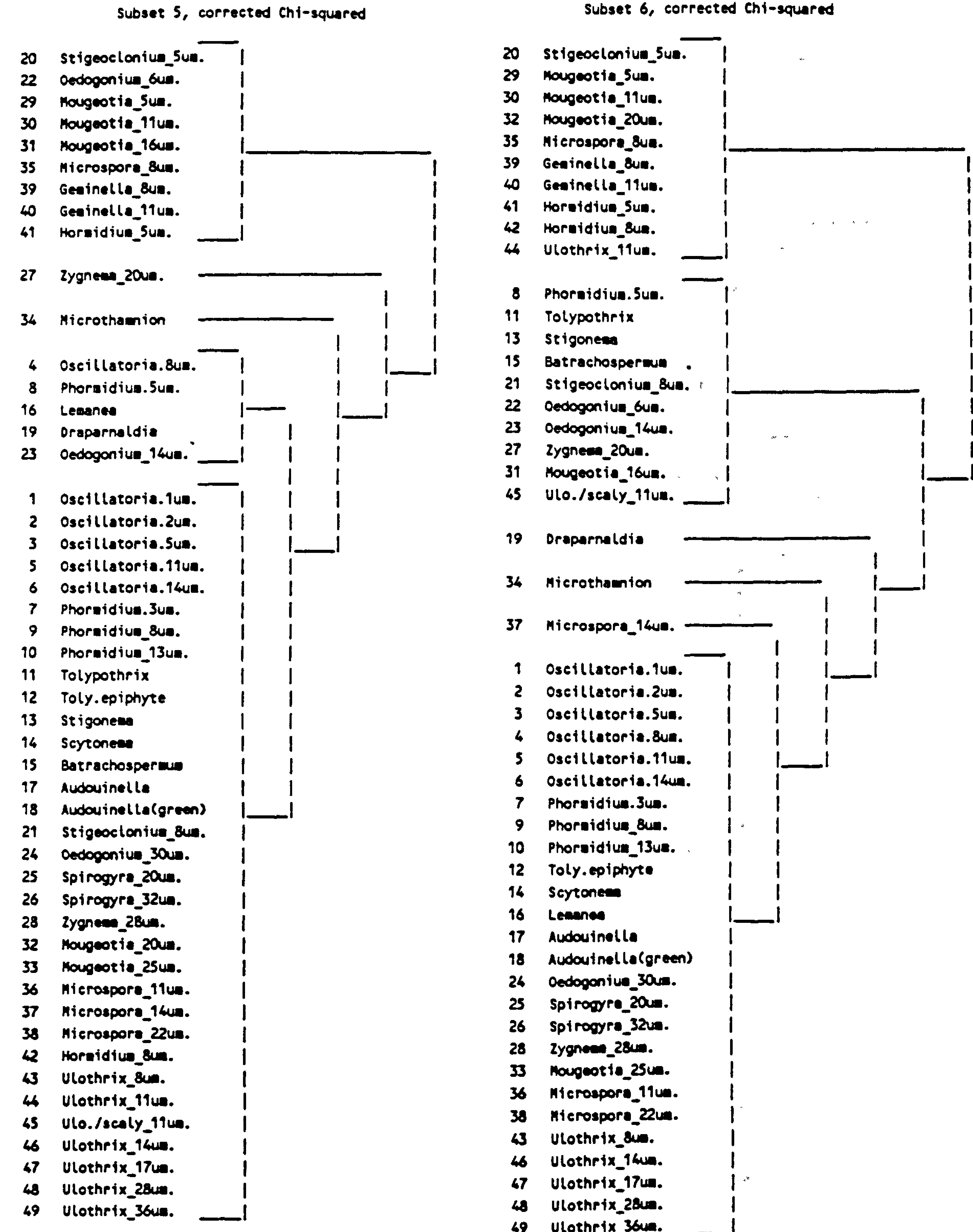


FIG.38

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

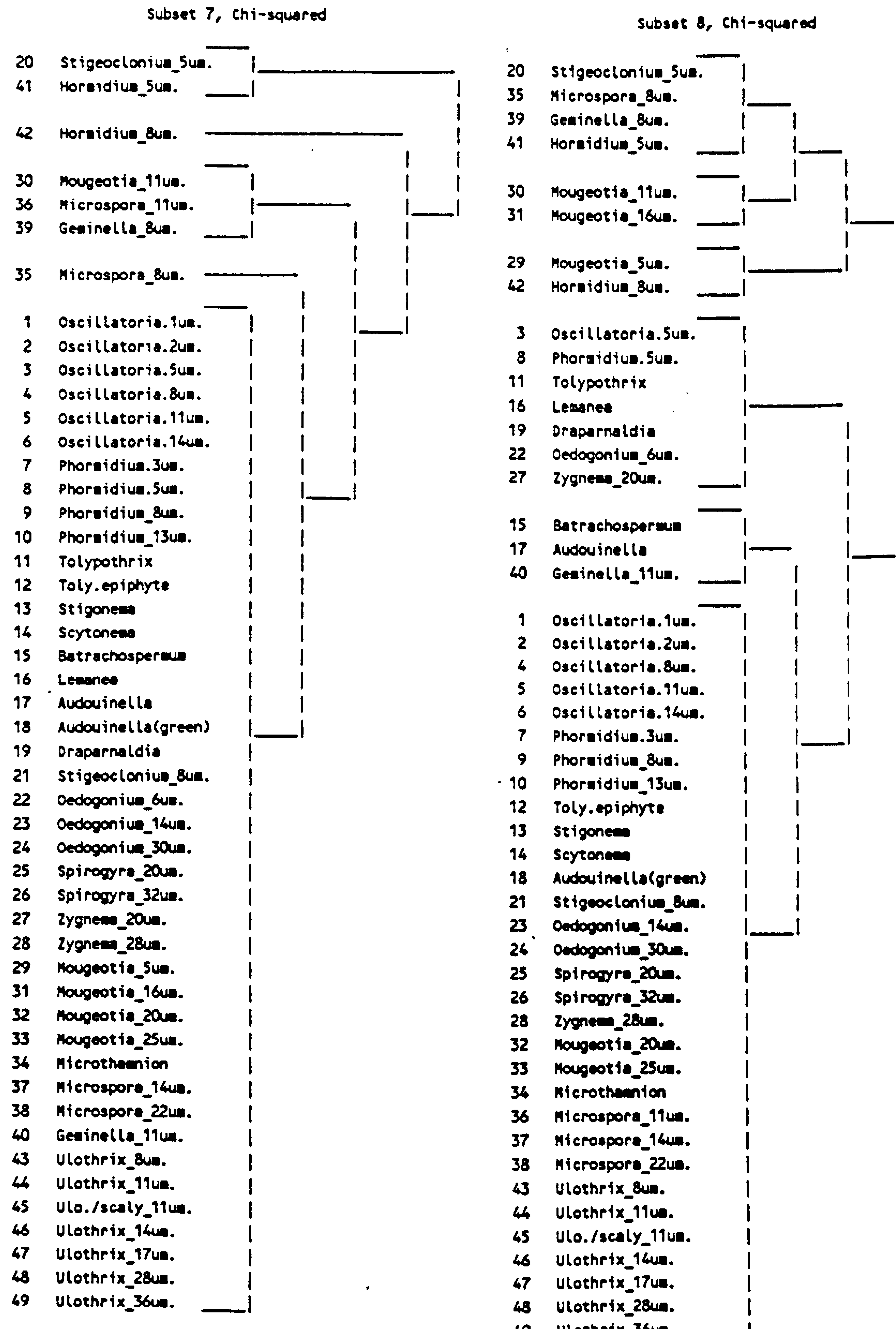


FIG.39

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

Subset 7, corrected Chi-squared

20 Stigeoclonium_5um.
41 Hormidium_5um.

42 Hormidium_8um.

30 Mougeotia_11um.

36 Microspora_11um.

39 Geminella_8um.

31 Mougeotia_16um.
43 Ulothrix_8um.

1 Oscillatoria.1um.
2 Oscillatoria.2um.
3 Oscillatoria.5um.
4 Oscillatoria.8um.
5 Oscillatoria.11um.
6 Oscillatoria.14um.
7 Phormidium.3um.
8 Phormidium.5um.
9 Phormidium_8um.
10 Phormidium_13um.
11 Tolypothrix
12 Toly.epiphyte
13 Stigonema
14 Scytonema
15 Batrachospermum
16 Lemanea
17 Audouinella
18 Audouinella(green)
19 Draparnaldia
21 Stigeoclonium_8um.
22 Oedogonium_6um.
23 Oedogonium_14um.
24 Oedogonium_30um.
25 Spirogyra_20um.
26 Spirogyra_32um.
27 Zygnema_20um.
28 Zygnema_28um.
29 Mougeotia_5um.
32 Mougeotia_20um.
33 Mougeotia_25um.
34 Microthamnion
35 Microspora_8um.
37 Microspora_14um.
38 Microspora_22um.
40 Geminella_11um.
44 Ulothrix_11um.
45 Ulo./scaly_11um.
46 Ulothrix_14um.
47 Ulothrix_17um.
48 Ulothrix_28um.
49 Ulothrix_36um.

Subset 8, corrected Chi-squared

20 Stigeoclonium_5um.
29 Mougeotia_5um.
30 Mougeotia_11um.
31 Mougeotia_16um.
35 Microspora_8um.
39 Geminella_8um.
41 Hormidium_5um.
42 Hormidium_8um.

3 Oscillatoria.5um.
8 Phormidium.5um.
11 Tolypothrix
16 Lemanea
19 Draparnaldia
22 Oedogonium_6um.
27 Zygnema_20um.

15 Batrachospermum
17 Audouinella
40 Geminella_11um.

1 Oscillatoria.1um.
2 Oscillatoria.2um.
4 Oscillatoria.8um.
5 Oscillatoria.11um.
6 Oscillatoria.14um.
7 Phormidium.3um.
9 Phormidium_8um.
10 Phormidium_13um.
12 Toly.epiphyte
13 Stigonema
14 Scytonema
18 Audouinella(green)
21 Stigeoclonium_8um.
23 Oedogonium_14um.
24 Oedogonium_30um.
25 Spirogyra_20um.
26 Spirogyra_32um.
28 Zygnema_28um.
32 Mougeotia_20um.
33 Mougeotia_25um.
34 Microthamnion
36 Microspora_11um.
37 Microspora_14um.
38 Microspora_22um.
43 Ulothrix_8um.
44 Ulothrix_11um.
45 Ulo./scaly_11um.
46 Ulothrix_14um.
47 Ulothrix_17um.
48 Ulothrix_28um.
49 Ulothrix_36um.

FIG.40

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC

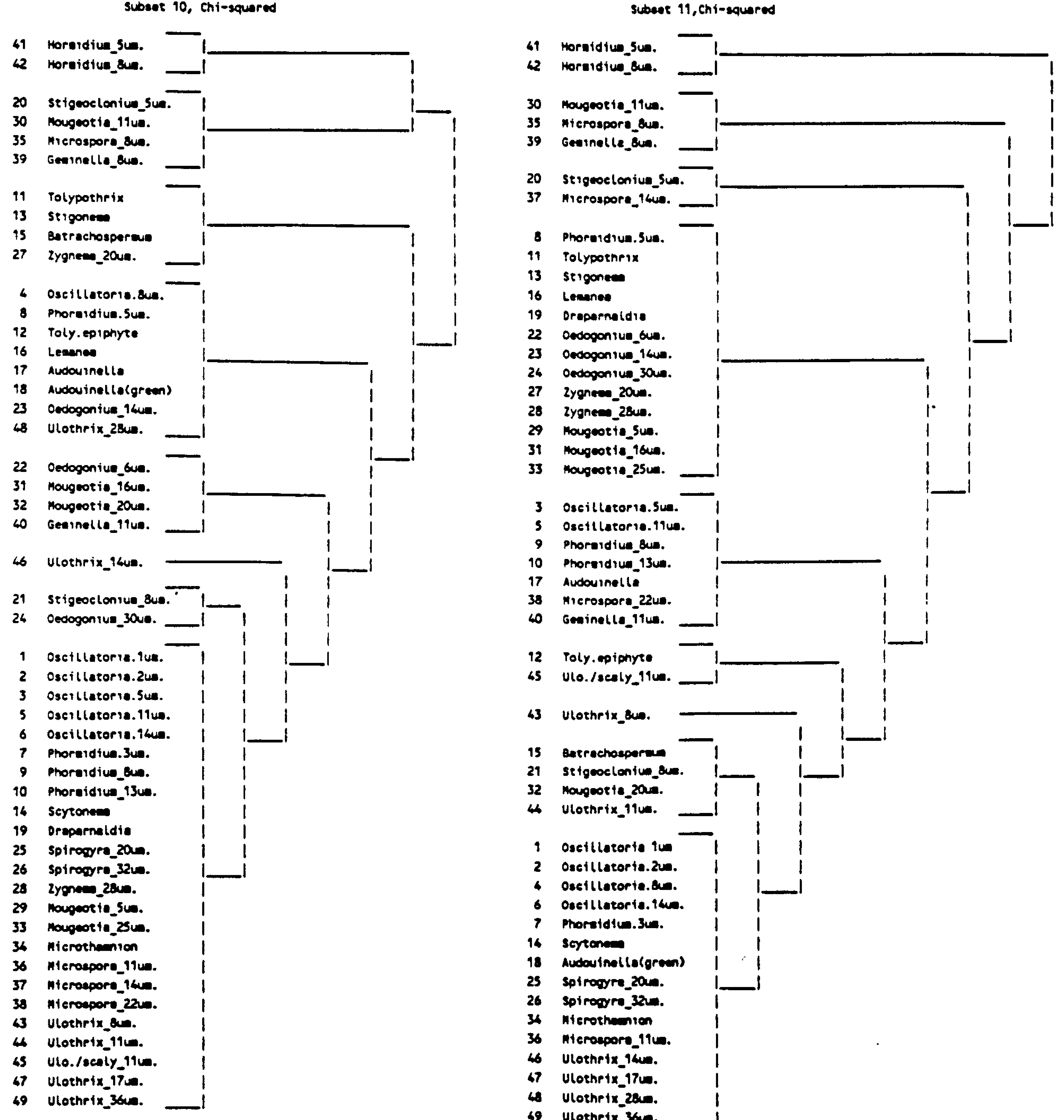
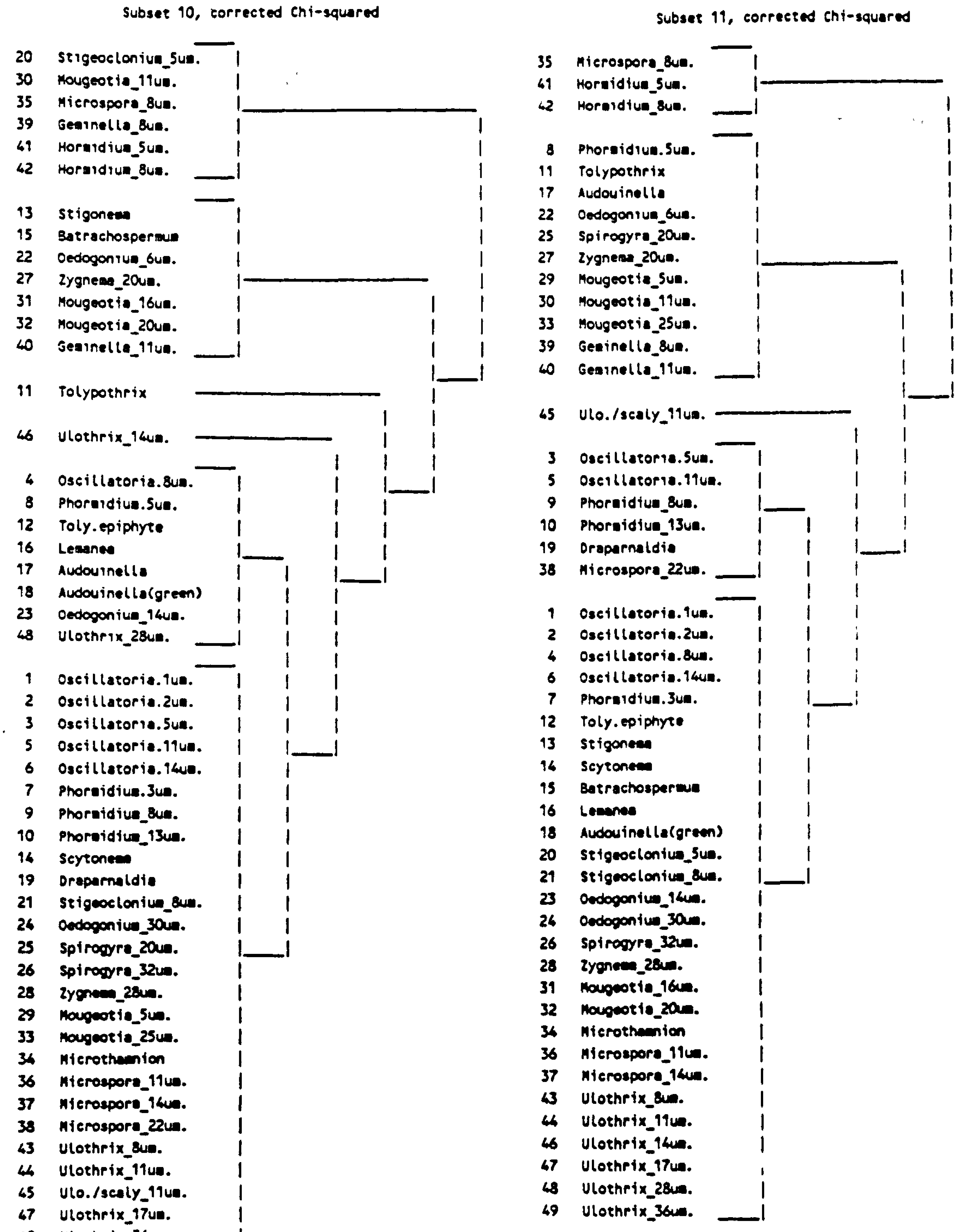


FIG.41

INVERSE ASSOCIATION ANALYSIS

Species Associations by NASSOC



Therefore reviewing all the results obtained using Inverse Association Analysis with subsets, a broad consensus appears about the existence of a group of species occurring in the more acidified sites, which may be considered acidobiontic. However it appears that the analysis is very sensitive to the quality of the data used. Inclusion of a large number of samples in the formation of the presence-absence matrix greatly improves the consistency of the results. It is also clear that the greater the number of sites included in the analysis the better the results will be since it is on the basis of the number of presences or absences of each species in sites that the χ^2 values are calculated.

In Normal Association Analysis the results are likely to be more robust since the divisions are on the basis of presence-absence of a sites in 49 different species, rather than onthe basis of each species in 15 (or less) sites in IAA.

4.6 EVIDENCE FOR COMMUNITY DIFFERENCES BETWEEN SITES

The hypothesis that the abundances of different species are influenced by environmental factors is implicit in the use of species diversity as an index of ecosystem disturbance. At the level of the individual species, each is considered to have an optimum range of values of any environmental variable, within which it will achieve the greatest rate of increase, and hence the highest abundance. The abundances of this species in different locations may thus be taken as an indicator of the values of that variable in each location. However species are influenced by more than one variable at a time and thus one species may give uncertain information about a variable. Use of several species simultaneously, or whole communities, is therefore likely to give a better indication of the likely value of one (or more) environmental variables. For this to be valid, distinct communities of organisms must be found to exist in sites which differ with regard to the environmental variable under consideration.

Statistical methods of assessing the similarity between communities have been devised (reviewed in Ludwig and Reynolds, 1988). The different approaches may be seen as means of assessing the degree of association between species (i.e. in communities) based on their (co-)occurrence in different Sampling Units, or of association between SUs (sites, in this work) on the basis of the species found in them. A series of tests is suggested by Ludwig and Reynolds (1988) for both abundance and presence-absence data, to analyse interspecific relationships and subsequently to classify the communities. Later stages in the testing, involving Correspondence Analysis, Polar Ordination or Principal Components Analysis have not been utilized, Canonical Correspondence Analysis by the Program CANOCO (Ter Braak, 1988) being preferred.

Abundance data may be used to test for covariance between species, with the recommendations that if species are not normally distributed the abundances should be ranked, and that the elimination of double-zero matches may be necessary. Species covariations may be calculated using the program SPCOVAR.BAS (Ludwig and Reynolds, 1988) but it does not include the option for the latter manipulation. Instead therefore species covariation (correlation) was carried out in MINITAB using a purpose-written macro to remove double-zero matches from each species pair in turn.

Presence-absence data may be used to compute indices of SU similiarity using the program SPASSOC.BAS (Ludwig and Reynolds, 1988). Presence-absence data are also used in Normal Association Analysis, carried out by NASSOC.BAS (Ludwig and Reynolds, 1988), which classifies the sites by the algal communities they contain.

Community classification may also be carried out using Cluster Analysis by the program CLUSTER.BAS (Ludwig and Reynolds, 1988), using abundance data.

Both Normal Association Analysis and Cluster Analysis allow the construction of dendrograms which graphically summarise the relationships between SUs and species, and are more readily interpretable than tables of numbers.

4.6.1 Species Covariation

Similarity between sites has been investigated by calculation of correlations between mean species abundance values in pairs of sites (Table 42). Values of **r** shown are the Pearson correlation coefficient in the upper half of the table, and Spearman ranked correlations in the lower half. This procedure is analogous to the calculation of species covariance. Double-zero matches have been eliminated from both sets of calculations as recommended by Ludwig and Reynolds (1988). They also recommend the use of the Spearman correlation if the species abundances are not normally distributed as required for the validity of Pearson's correlation coefficient. Normal probability correlations carried out in MINITAB reveal that most species fail the test for normal distribution at the 1% level. Table 42 shows the correlations, the number of data points from which **r** was determined, and the value **p** (probability level) of the regression. With 105 separate correlations in each half, an appropriate level of significance is 0.5% ($p < 0.005$).

Considering the Spearman correlations, the majority of high values of **r** (> approx. 0.5) have a **p** value of 0.005 or less and may thus be considered significantly different from zero. Large positive correlations occur between sites 14-1, 14-2, 2, 10-1 and 10-2; sites 15 and 6; sites 2, 5 and 11-2; sites 5, 6, and 10-2; sites 6 and 5/6; and between most pairs of sites in the group 9, 10-1, 10-2, 11-1 and 11-2, where the largest correlations occur. Lower but significant positive correlations occur between sites C-1 and C-2, and negative between sites Ward and 2,11-1 and 11-2.

The Pearson correlation yields higher values for these sites and includes a larger number of significant correlations, but must be considered less trustworthy as discussed above.

TABLE 42

SAMPLING UNITS COVARIANCE MATRIX BASED ON SPECIES (RELATIVE) ABUNDANCE DATA

(a) DOUBLE-ZERO MATCHES ELIMINATED

BURNS/SITES		14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
14-1	r		0.918	0.175	0.883	0.805	0.351	0.348	0.559	0.731	0.729	0.460	0.673	0.382	0.088	-0.097
	n		23	38	28	35	40	28	24	25	23	23	24	40	43	40
	p		<0.001	0.300	<0.001	<0.001	0.030	0.070	0.005	<0.001	<0.001	0.030	<0.001	0.015	0.570	0.550
14-2	r	0.698		0.276	0.822	0.774	0.347	0.389	0.686	0.841	0.872	0.414	0.833	0.384	0.007	-0.097
	n	23		37	27	31	40	29	19	22	19	18	19	38	43	41
	p	<0.001		0.001	<0.001	<0.001	0.030	0.040	0.001	<0.001	<0.001	0.090	<0.001	0.017	0.960	0.550
15	r	0.064	-0.083		0.089	0.087	0.439	0.155	0.176	0.242	0.317	-0.001	0.240	0.445	0.346	0.258
	n	38	37		39	41	42	39	38	38	37	38	38	44	47	44
	p	0.700	0.620		0.590	0.590	0.004	0.350	0.290	0.143	0.056	1.000	0.150	0.003	0.017	0.090
2	r	0.573	0.549	-0.064		0.868	0.407	0.526	0.528	0.680	0.678	0.452	0.672	0.262	0.033	-0.173
	n	28	27	39		35	41	31	28	29	27	28	27	42	45	44
	p	0.001	0.003	0.700		<0.001	0.008	0.002	0.004	<0.001	<0.001	0.016	<0.001	0.094	0.830	0.260
5	r	0.395	0.375	0.075	0.556		0.502	0.513	0.702	0.720	0.677	0.430	0.689	0.187	0.020	-0.176
	n	35	31	41	35		41	35	31	31	30	31	31	39	42	42
	p	0.019	0.038	0.640	0.001		0.001	0.002	<0.001	<0.001	<0.001	0.016	<0.001	0.250	0.900	0.265
6	r	0.236	0.141	0.481	0.358	0.551		0.705	0.277	0.346	0.342	0.118	0.291	0.260	0.156	-0.080
	n	40	40	42	41	41		40	42	41	40	41	41	45	46	44
	p	0.143	0.390	0.001	0.022	<0.001		<0.001	0.075	0.027	0.030	0.460	0.065	0.085	0.300	0.610
5/6	r	0.024	0.048	0.034	0.404	0.439	0.632		0.305	0.366	0.394	0.070	0.386	0.122	-0.073	-0.146
	n	28	29	39	31	35	40		29	29	28	28	29	40	44	43
	p	0.900	0.810	0.840	0.024	0.008	<0.001		0.107	0.051	0.038	0.720	0.039	0.450	0.640	0.350
9	r	0.404	0.536	-0.131	0.347	0.318	0.065	-0.120		0.982	0.845	0.363	0.709	0.239	-0.081	-0.111
	n	24	19	38	28	31	42	29		21	18	19	18	37	42	41
	p	0.050	0.180	0.430	0.070	0.081	0.680	0.534		<0.001	<0.001	0.127	0.001	0.155	0.610	0.490
10-1	r	0.540	0.629	-0.052	0.457	0.467	0.216	-0.004	0.811		0.975	0.539	0.833	0.385	-0.038	-0.117
	n	25	22	38	29	31	41	29	21		20	21	22	38	42	41
	p	0.005	0.002	0.760	0.013	0.008	0.174	0.980	<0.001		<0.001	0.012	<0.001	0.017	0.810	0.470
10-2	r	0.692	0.600	-0.080	0.482	0.508	0.148	-0.053	0.780	0.868		0.432	0.828	0.449	-0.014	-0.070
	n	23	19	37	27	30	40	28	18	20		19	19	37	42	39
	p	<0.001	0.007	0.640	0.011	0.004	0.360	0.790	<0.001	<0.001		0.065	<0.001	0.005	0.930	0.670
11-1	r	0.461	0.386	-0.161	0.457	0.286	0.143	-0.034	0.597	0.758	0.709		0.592	0.070	-0.122	-0.244
	n	23	18	38	28	31	41	29	18	22	19		19	38	42	40
	p	0.027	0.114	0.330	0.015	0.119	0.370	0.860	0.007	<0.001	0.001		0.008	0.676	0.440	0.130
11-2	r	0.442	0.458	-0.116	0.601	0.345	0.191	0.025	0.738	0.795	0.723	0.853		0.282	-0.075	-0.111
	n	24	19	38	27	31	41	29	18	22	19	19		38	42	41
	p	0.031	0.050	0.490	0.001	0.057	0.230	0.900	<0.001	<0.001	<0.001	<0.001		0.090	0.640	0.490
C-1	r	-0.066	-0.006	0.124	-0.079	0.166	0.153	-0.167	0.049	-0.062	0.106	-0.142	-0.102		0.621	0.321
	n	40	38	44	42	39	45	40	37	38	37	38	38		42	44
	p	0.690	0.970	0.420	0.620	0.312	0.320	0.300	0.780	0.710	0.533	0.395	0.541		<0.001	0.032
C-2	r	0.122	-0.104	0.248	-0.033	0.147	0.303	-0.082	-0.082	-0.101	-0.019	-0.094	-0.042	0.454		0.566
	n	43	43	47	45	42	46	44	42	42	42	42	42	42		45
	p	0.440	0.510	0.090	0.830	0.350	0.040	0.600	0.610	0.520	0.910	0.552	0.790	0.003		<0.001
WARD	r	-0.284	-0.437	0.108	-0.427	-0.183	-0.231	-0.345	-0.347	-0.385	-0.398	-0.520	-0.444	0.104	0.314	
	n	40	41	44	44	42	44	43	41	41	39	40	41	44	45	
	p	0.076	0.004	0.490	0.004	0.245	0.131	0.024	0.026	0.013	0.012	0.001	0.004	0.500	0.036	

(b) DOUBLE ZERO MATCHES ELIMINATED, RANKED

4.6.2 Species Association

Similarity between sites is also shown in Tables 43-45 compiled using the program SPASSOC.BAS (Ludwig and Reynolds, 1988), which computes similarity indices based on species presence-absence. The tables show values of the Ochiai and Jaccard indices. Only values occurring in site pairs showing a significant χ^2 value (> 3.84) have been included. Significant values are seen to occur between upstream-downstream pairs of sites, and within the group of sites which may be described as the most acidified, having the lowest mean pH values (Table 46). These sites are on burns 2, 5, 9, 10, 11 and 14. The results taken with those of species covariation therefore support the idea that such an 'acidified group' of sites does exist. Comparison of the values of both indices in the three data matrices A, B and C show no clear tendency for values to be higher in one matrix.

This analysis being based on p/a data is free of any criticism of bias due to estimation of relative abundance.

4.6.3 Normal Association Analysis

The testing sequence suggested for presence-absence data matrices utilizes the program NASSOC.BAS to perform Normal Association Analysis. The first stage of the analysis involves the Variance Ratio test for significant association between multiple species, and all data sets used have passed this test. (Normal) Association Analysis determines a χ^2 value for similarity between pairs of Sampling Units on the basis of the species they contain, and sorts the SUs into homologous groups using these χ^2 values. Homology is achieved when no χ^2 value greater than 3.84 is found. If the procedure is carried out with an inverted data matrix species may be sorted into groups on the basis of the SUs in which they occur. This procedure is referred to as Inverse Association Analysis (see Section 4.4.2).

The results of the analyses are shown in the form of dendrograms in Figs. 42 and 43. Ludwig and Reynolds (1988) recommend removal of rare species from the data in order to minimise bias. This has been carried out in Figs. 44 and 45.

The analyses carried out with the uncorrected values ultimately result in a large number of small groups of sites, while the corrected χ^2 gives only three groups. However the division arising at the first cycle is almost identical in all matrices and utilizing both χ^2 and

TABLE 43

S.U. Associations by SPASSOC.BAS; Matrix A.
Pairs showing corrected Chi-squared value >3.84

JACCARD'S INDEX

S.U.	S.U.	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
14-2			-	0.571	0.500	-	-	0.667	0.579	0.667	0.800	0.647	-	-	-
15		-		0.484	0.581	-	-	-	-	-	-	-	0.647	0.622	-
2		0.744	0.658		0.593	-	-	0.455	0.609	0.455	0.619	0.591	-	-	-
5		0.694	0.736	0.746		0.515	-	0.400	0.538	0.400	0.423	0.462	0.606	0.541	-
6		-	-	-	0.682		-	-	-	-	-	0.400	-	0.605	-
5/6		-	-	-	-	-		-	-	-	-	-	-	-	-
9		0.801	-	0.645	0.602	-	-		0.611	1.000	0.723	0.800	0.355	-	-
10-1		0.740	-	0.759	0.708	-	-	0.770		0.611	0.550	0.684	0.469	-	-
10-2		0.801	-	0.645	0.602	-	-	1.000	0.770		0.733	0.800	0.355	-	-
11-1		0.889	-	0.777	0.613	-	-	0.849	0.713	0.849		0.813	-	-	-
11-2		0.788	-	0.751	0.646	0.596	-	0.894	0.814	0.894	0.897		0.406	-	-
C-1		0.608	0.788	-	0.761	-	-	0.580	0.664	0.580	-	0.613		0.730	-
C-2		-	0.774	-	0.715	0.759	-	-	-	-	-	-	0.845		-
WARD		-	-	-	-	-	-	-	-	-	-	-	-	-	

OCHIAI INDEX

TABLE 44

S.U. Associations by SPASSOC.BAS; Matrix B.
Pairs showing corrected Chi-squared value >3.84

JACCARD'S INDEX

S.U.	S.U.	14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
14-1			0.571	-	0.643	-	-	0.571	0.375	0.435	0.545	0.478	0.522	-	-	-
14-2		0.744		-	0.500	0.370	-	0.423	0.529	0.529	0.588	0.500	0.474	-	-	-
15		-	-		0.556	-	0.737	-	-	-	-	-	-	-	-	-
2		0.789	0.707	0.716		0.563	0.590	0.613	0.393	0.393	0.481	0.429	0.519	-	-	-
5		-	0.566	-	0.721		0.622	0.548	0.370	0.480	0.520	0.462	-	-	-	-
6		-	-	0.852	0.752	0.782		0.579	-	-	-	-	-	-	-	-
5/6		0.730	0.623	-	0.761	0.708	0.748		-	0.370	0.407	0.462	0.444	-	-	-
9		0.558	0.692	-	0.598	0.566	-	-		0.625	0.588	0.500	0.556	-	-	-
10-1		0.620	0.692	-	0.598	0.679	-	0.566	0.769		0.688	0.800	0.474	-	-	-
10-2		0.717	0.741	-	0.681	0.709	-	0.600	0.741	0.815		0.647	0.526	-	-	-
11-1		0.657	0.667	-	0.629	0.655	-	0.655	0.667	0.889	0.786		0.611	-	-	-
11-2		0.693	0.645	-	0.709	-	-	0.632	0.716	0.645	0.690	0.759		-	-	-
C-1		-	-	-	-	-	-	-	-	-	-	-	-		-	-
C-2		-	-	-	-	-	-	-	-	-	-	-	-	-		0.762
WARD		-	-	-	-	-	-	-	-	-	-	-	-	-	0.865	

OCHIAI INDEX

TABLE 45

S.U. Associations by SPASSOC.BAS; Matrix C.
Pairs showing corrected Chi-squared value >3.84

JACCARD'S INDEX

S.U.	S.U.	14-1	14-2	15	2	5	6	5/6	9	10-1	10-2	11-1	11-2	C-1	C-2	WARD
14-1		-	0.585	-	0.643	-	-	0.607	0.458	0.600	0.565	0.565	0.500	-	-	-
14-2		0.727		-	0.556	0.452	-	0.414	0.632	0.636	0.684	0.778	0.684	-	-	-
15		-	-		-	-	0.714	-	-	-	-	-	-	-	-	-
2		0.789	0.735	-		0.571	-	0.645	0.464	0.586	0.556	0.500	0.556	-	-	-
5		-	0.650	-	0.728		0.634	0.543	0.419	0.581	0.500	0.452	0.452	0.667	0.690	-
6		-	-	0.835	-	0.783		0.575	-	-	-	-	-	-	-	-
5/6		0.760	0.600	-	0.784	0.706	0.746		-	0.552	0.464	0.464	0.414	-	-	-
9		0.635	0.775	-	0.658	0.623	-	-		0.667	0.772	0.632	0.722	-	-	-
10-1		0.750	0.783	-	0.745	0.747	-	0.716	0.808		0.800	0.714	0.636	-	-	-
10-2		0.727	0.813	-	0.735	0.696	-	0.650	0.839	0.894		0.684	0.684	-	-	-
11-1		0.727	0.875	-	0.686	0.650	-	0.650	0.775	0.839	0.813		0.684	-	-	-
11-2		0.671	0.813	-	0.735	0.650	-	0.600	0.839	0.783	0.813	0.813		-	-	-
C-1		-	-	-	-	0.805	-	-	-	-	-	-	-		0.857	-
C-2		-	-	-	-	0.831	-	-	-	-	-	-	-	0.926		-
WARD		-	-	-	-	-	-	-	-	-	-	-	-	-	-	

OCHIAI INDEX

TABLE 46

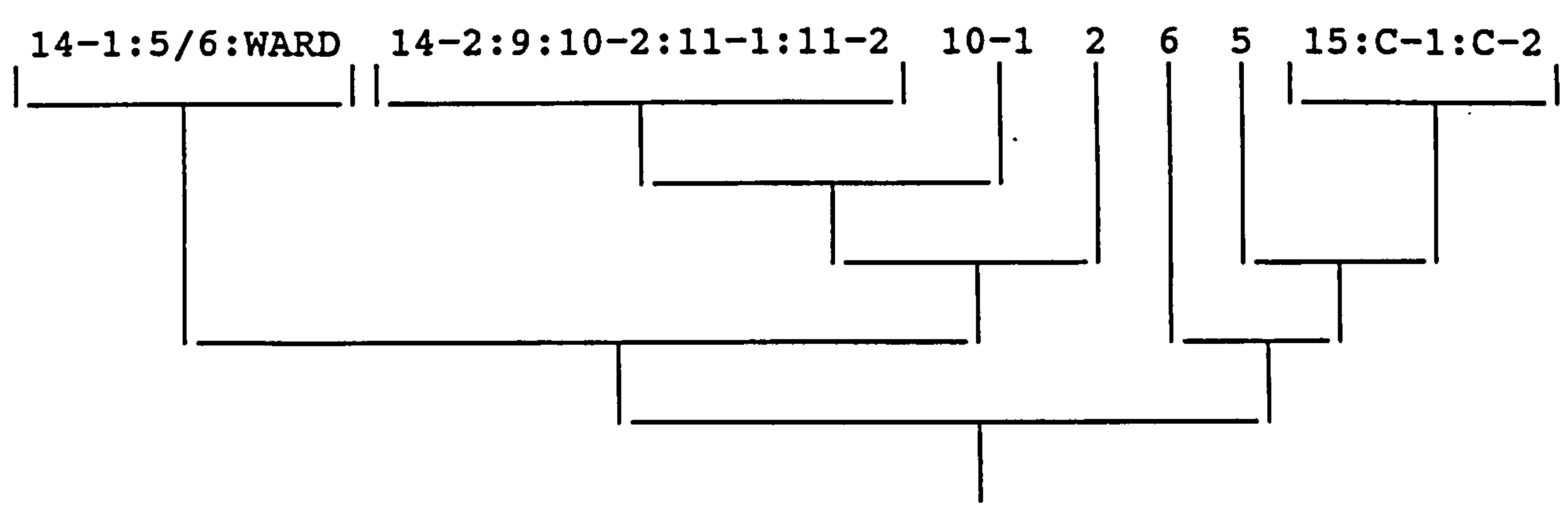
SAMPLING SITES, ARRANGED IN INCREASING ORDER OF MEAN pH, 1986-1988

SITE	9	11-2	11-1	10-1	10-2	14-2	14-1	2	5	5/6	6	15	C-1	C-2	WARD
MEAN pH	4.37	4.38	4.43	4.44	4.46	4.71	4.97	5.25	5.25	5.41	5.78	5.91	6.12	6.31	6.67

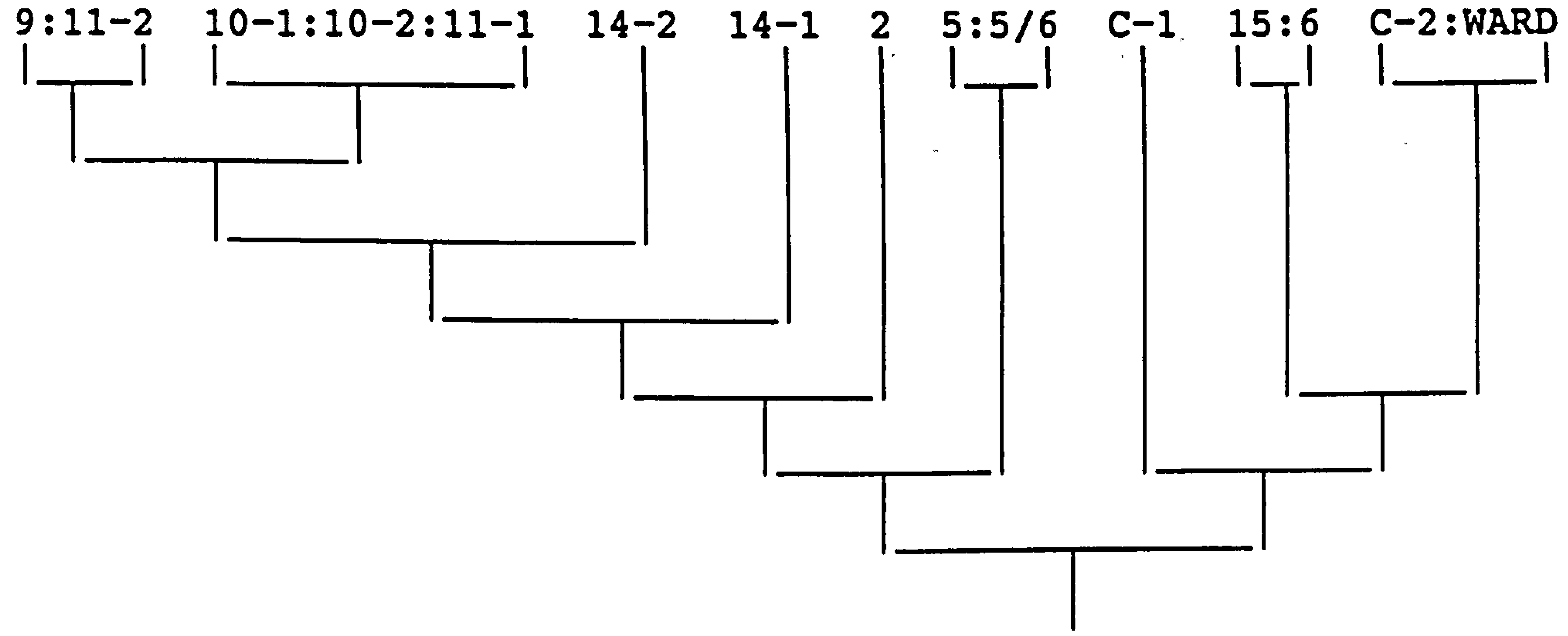
FIG.42

NASSOC Sampling Unit Associations:
Chi-squared on Presence-Absence data

Matrix A



Matrix B



Matrix C

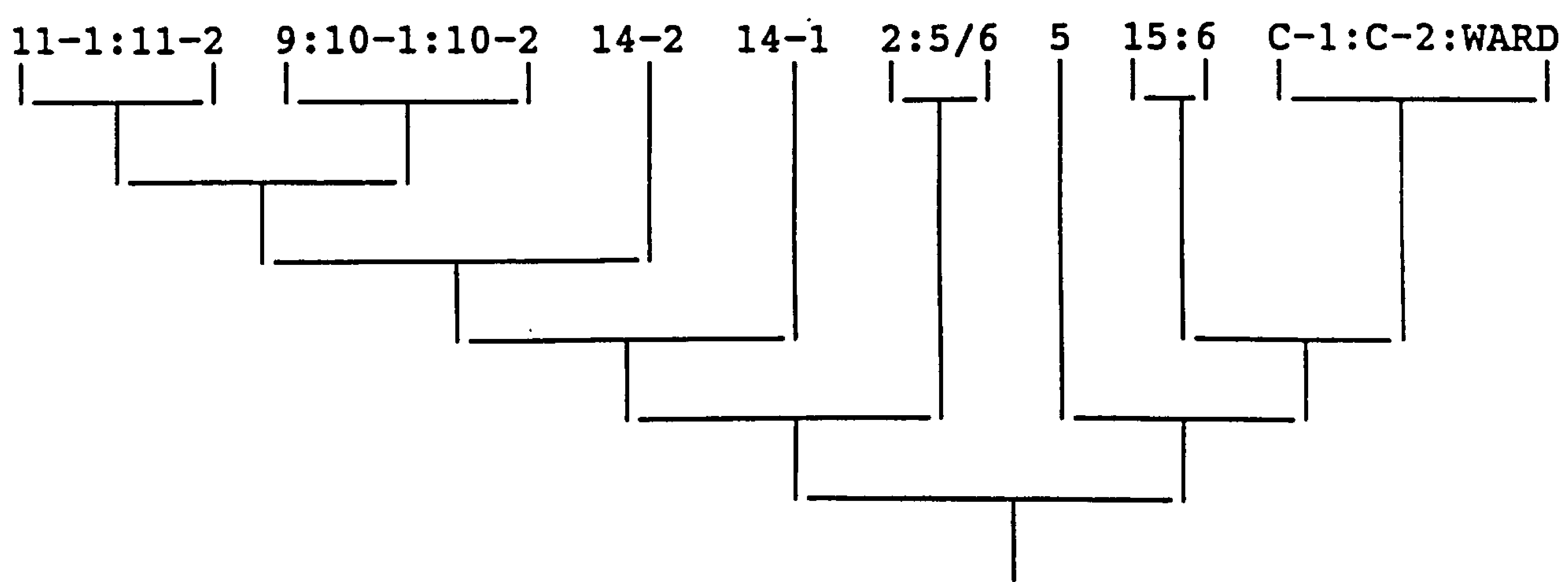


FIG.43

NASSOC Sampling Unit Associations:
Corrected Chi-squared on Presence-Absence data

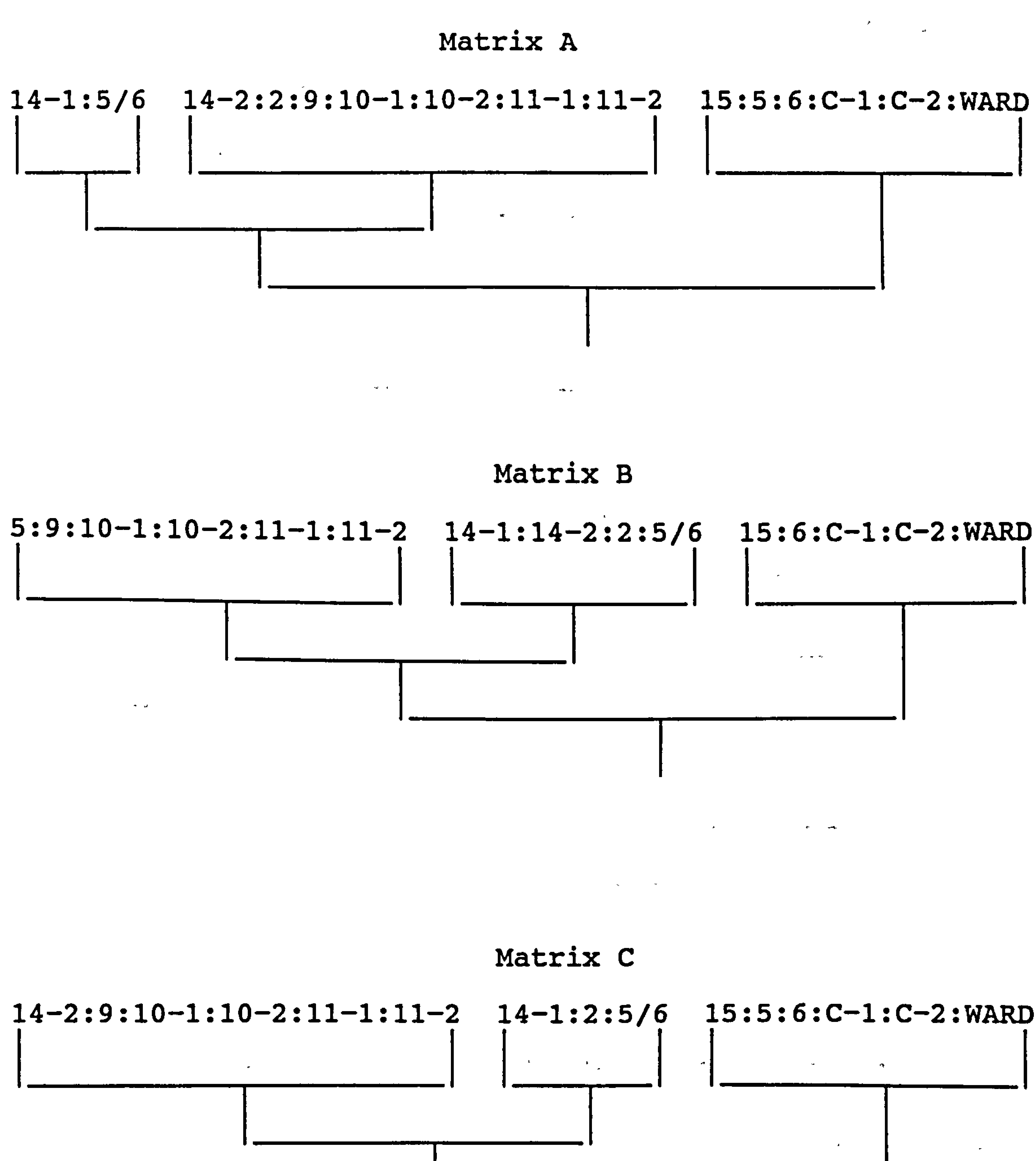


FIG.44

NASSOC Sampling Unit Associations:
Chi-squared on Presence-Absence data, rare species deleted

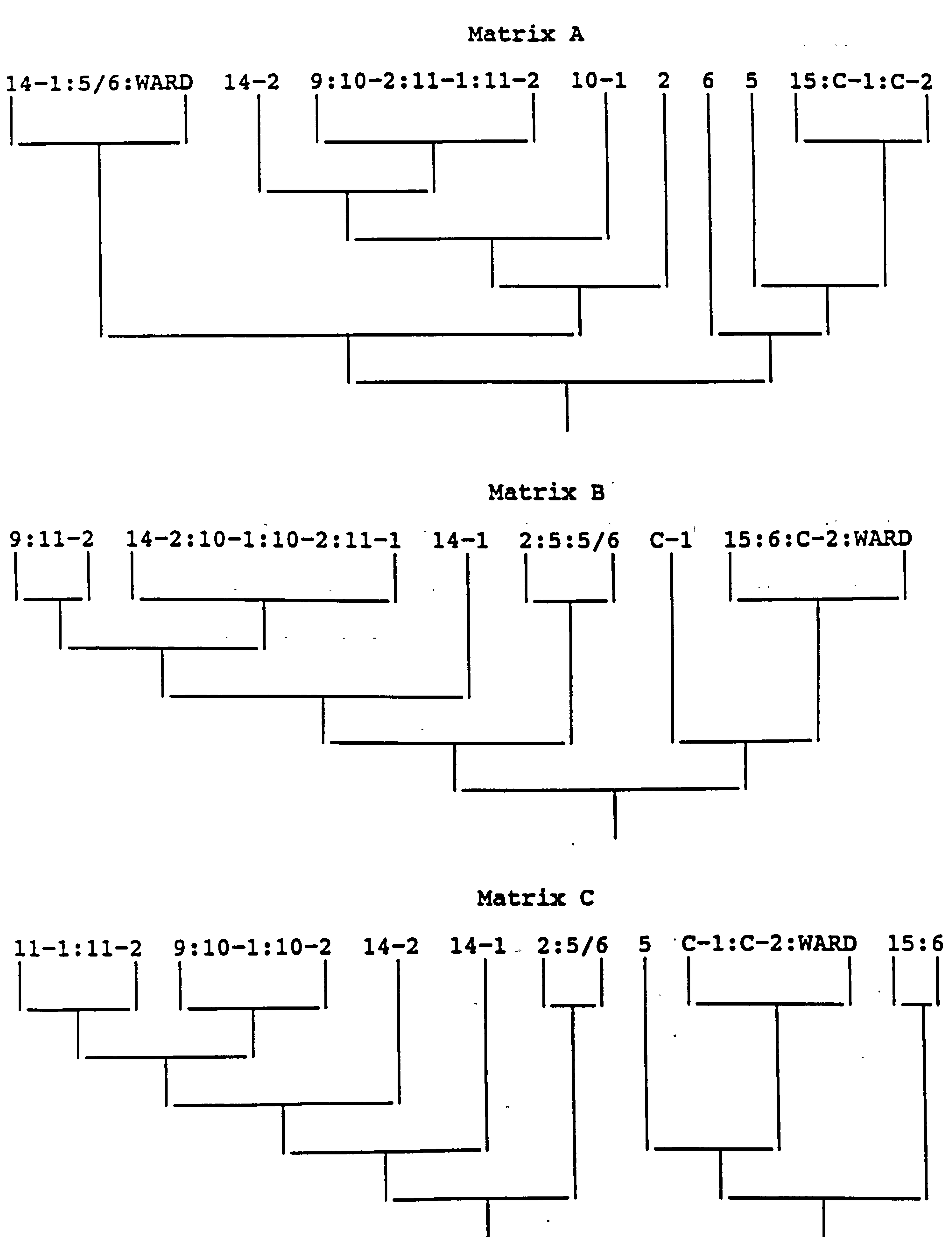
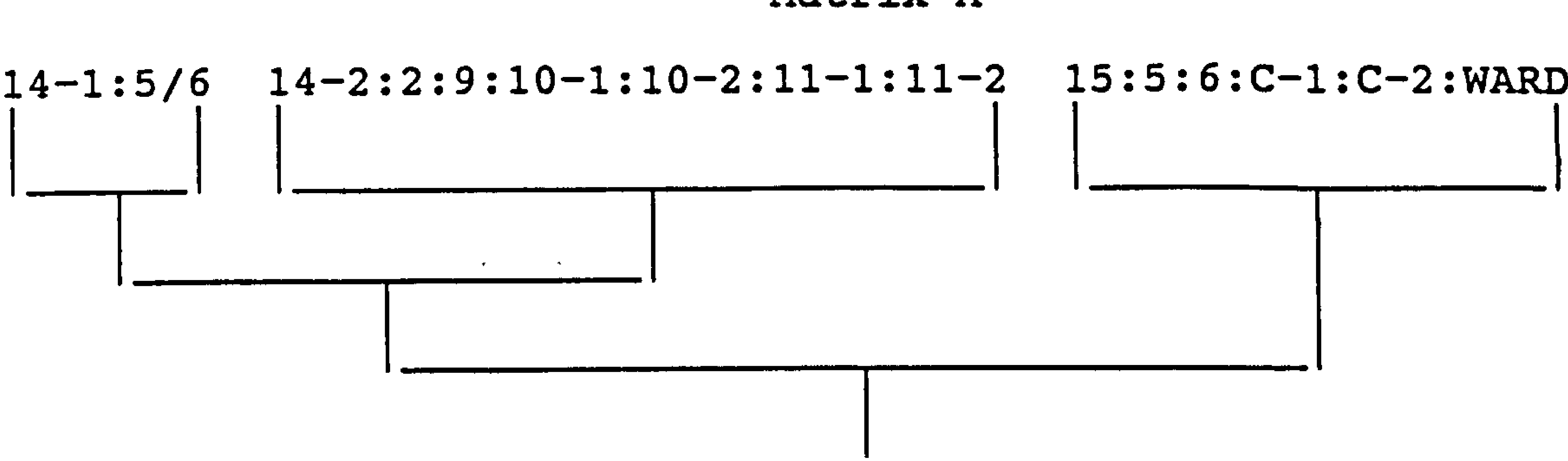


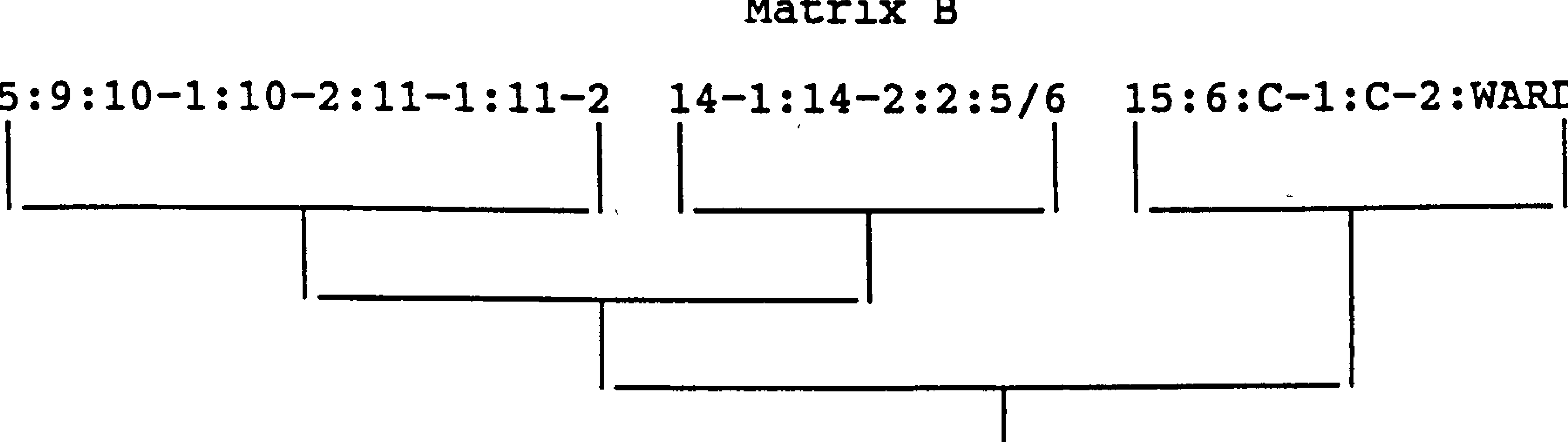
FIG.45

NASSOC Sampling Unit Associations:
Corrected Chi-squared on Presence-Absence data, rare species deleted

Matrix A



Matrix B



Matrix C

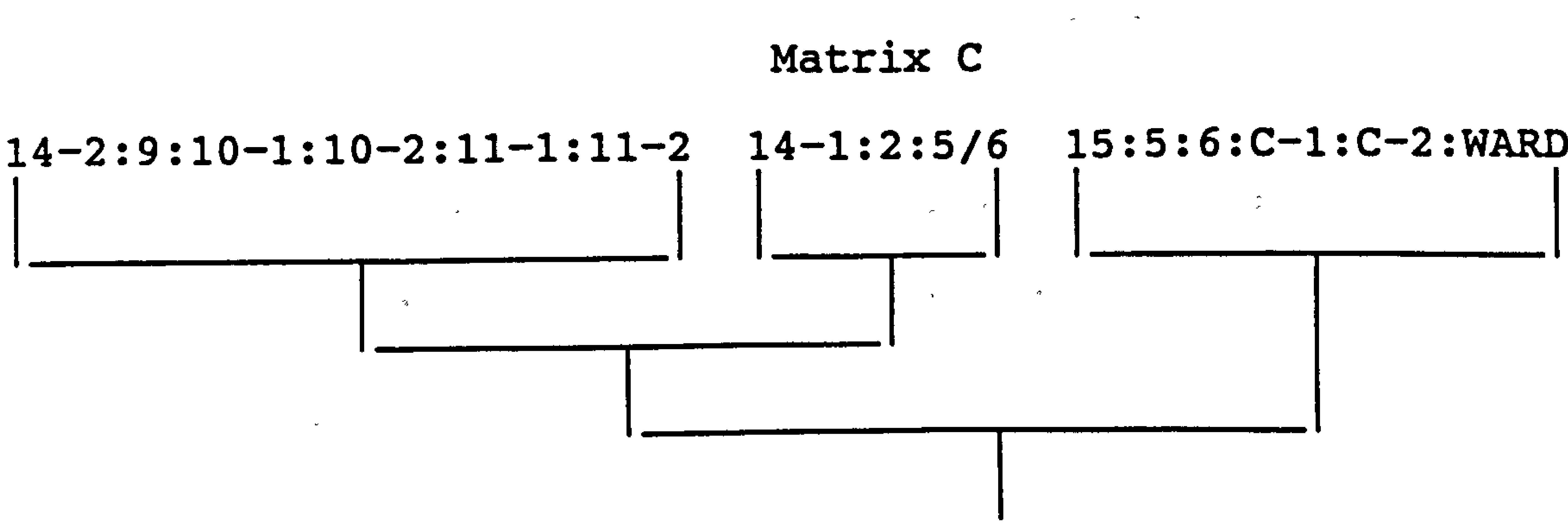
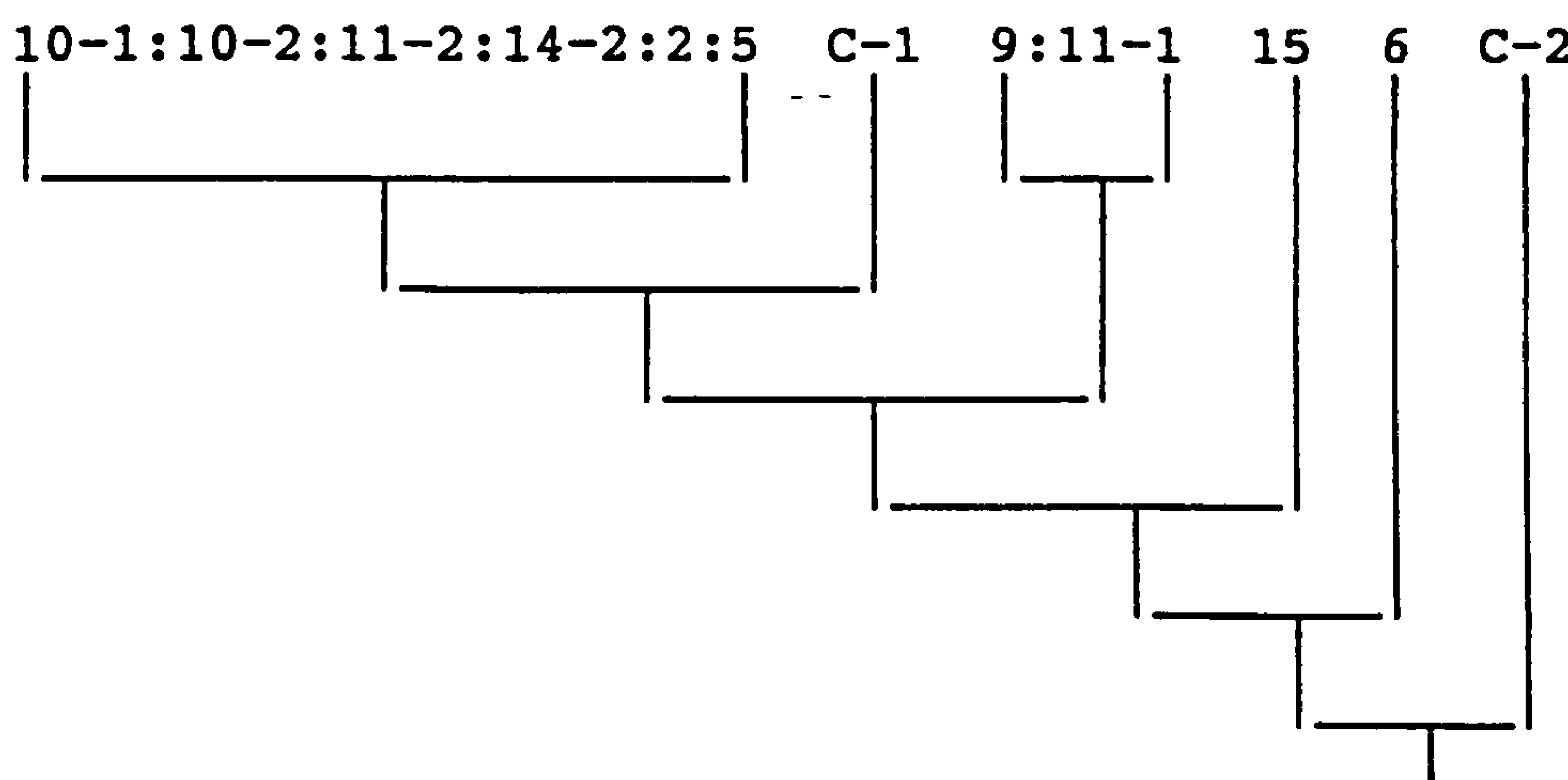


FIG.46

NASSOC Sampling Unit Associations:
Subsets 1 and 2 compared

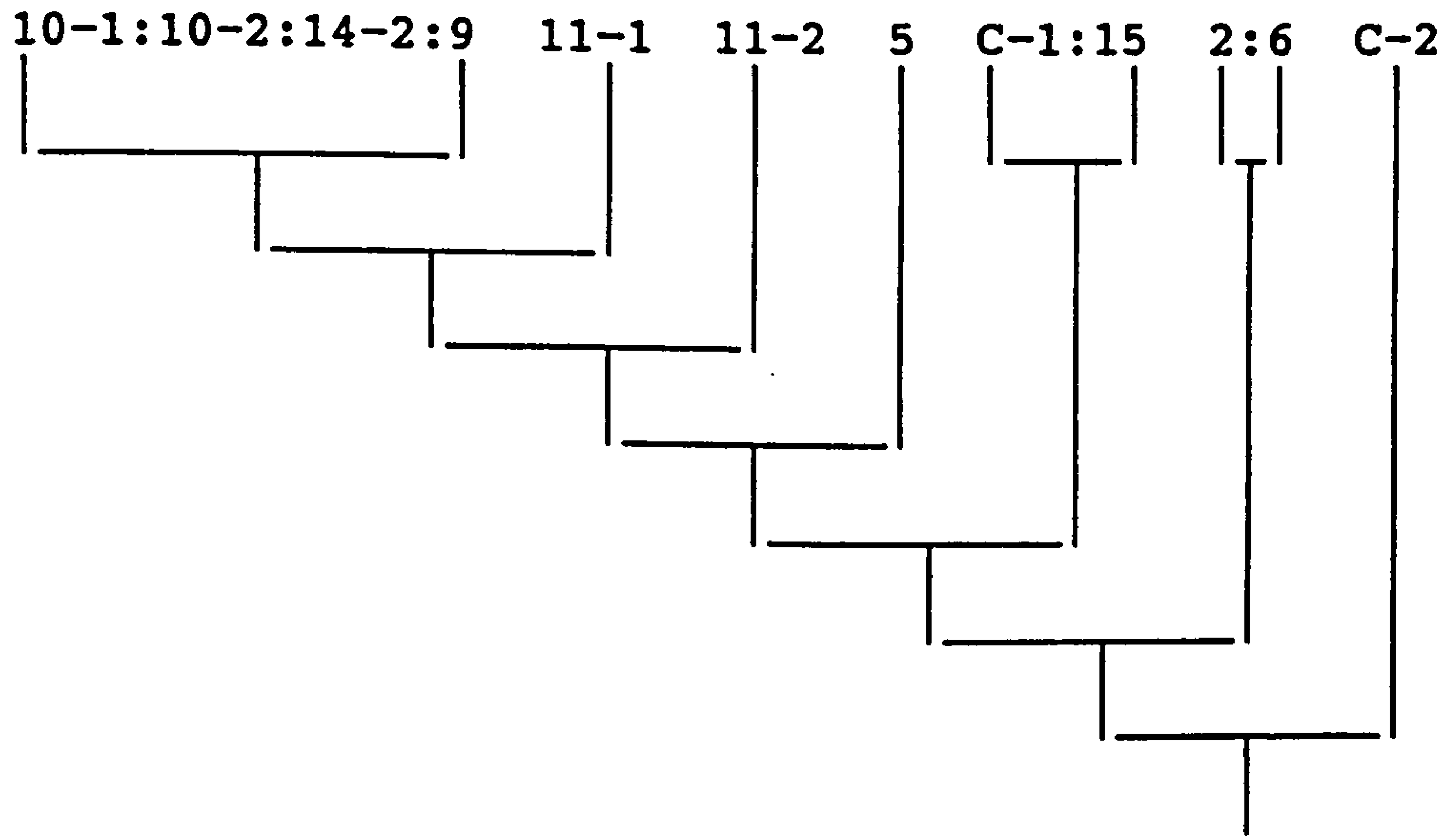
Subset 1, Chi-squared



Subset 1, corrected Chi-squared

No divisions performed

Subset 2, Chi-squared



Subset 2, corrected Chi-squared

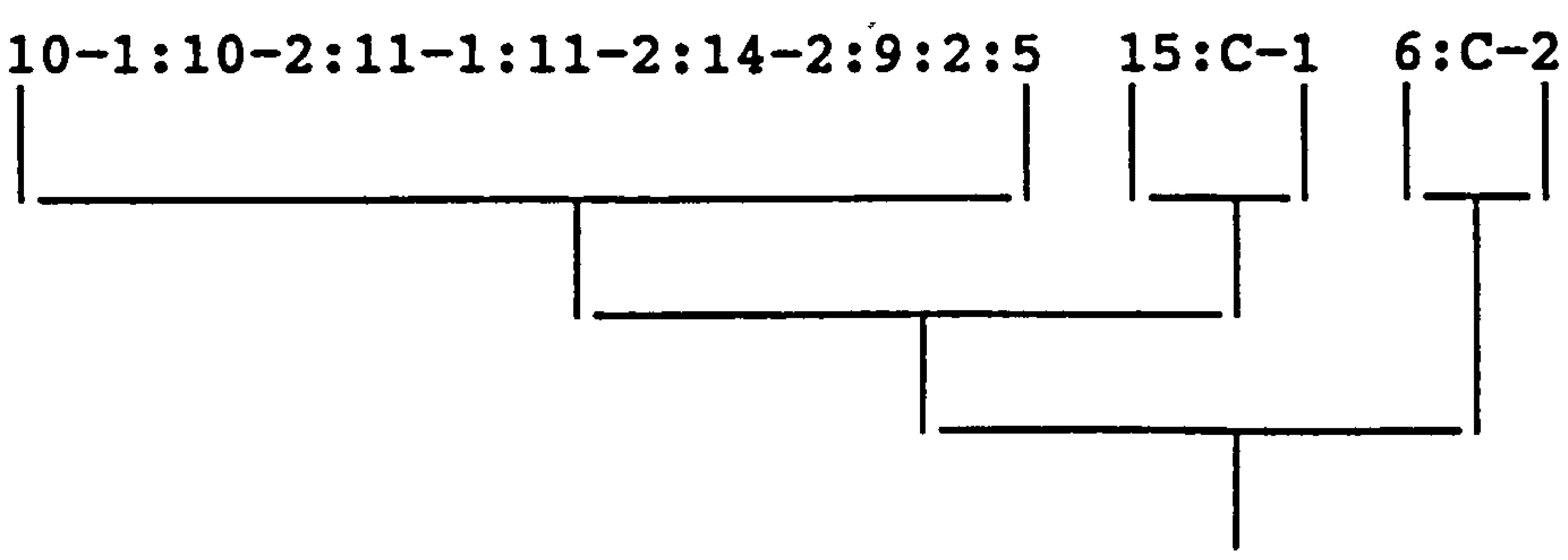
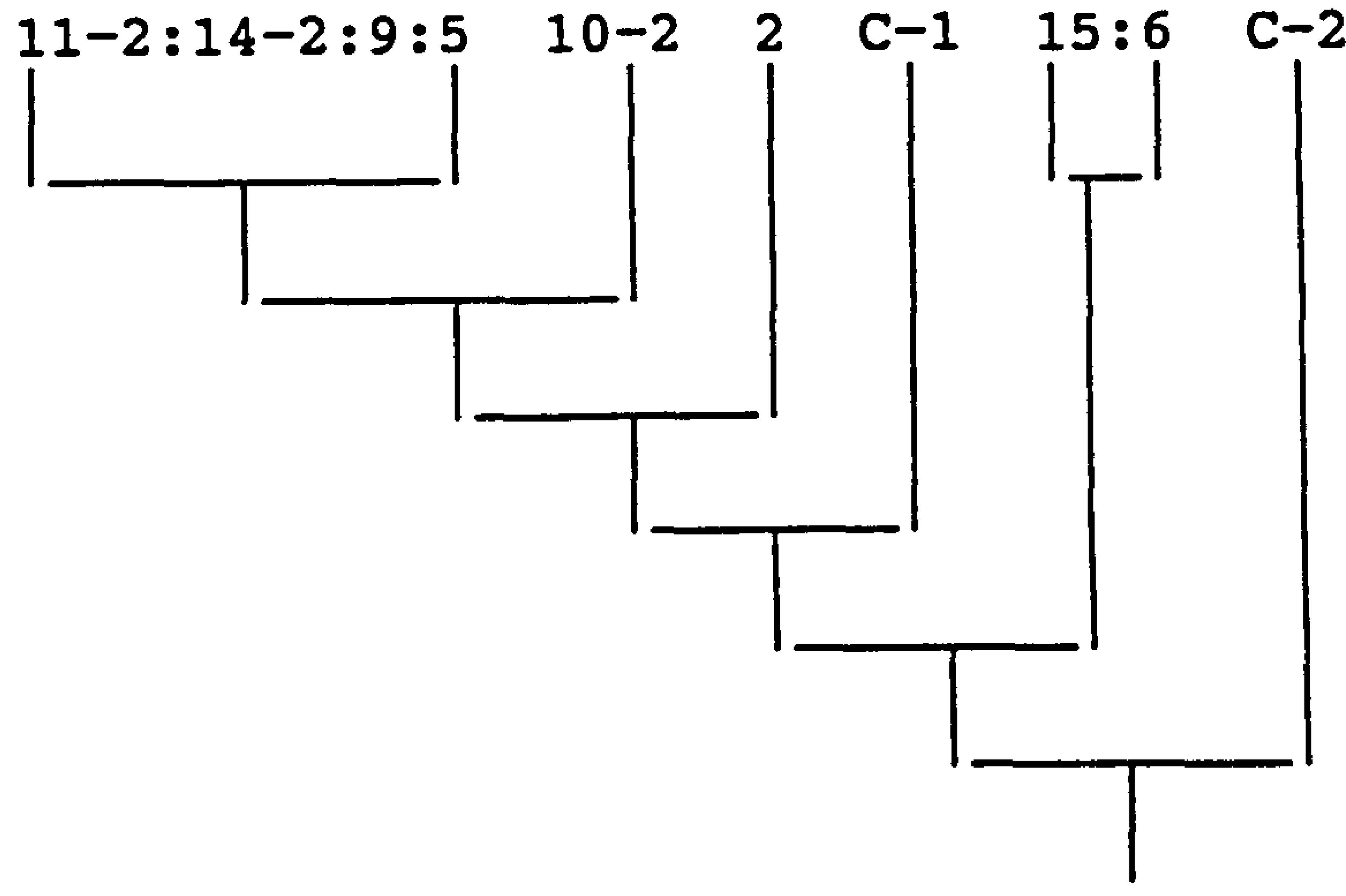


FIG.47

NASSOC Sampling Unit Associations:
Subsets 3 and 4 compared

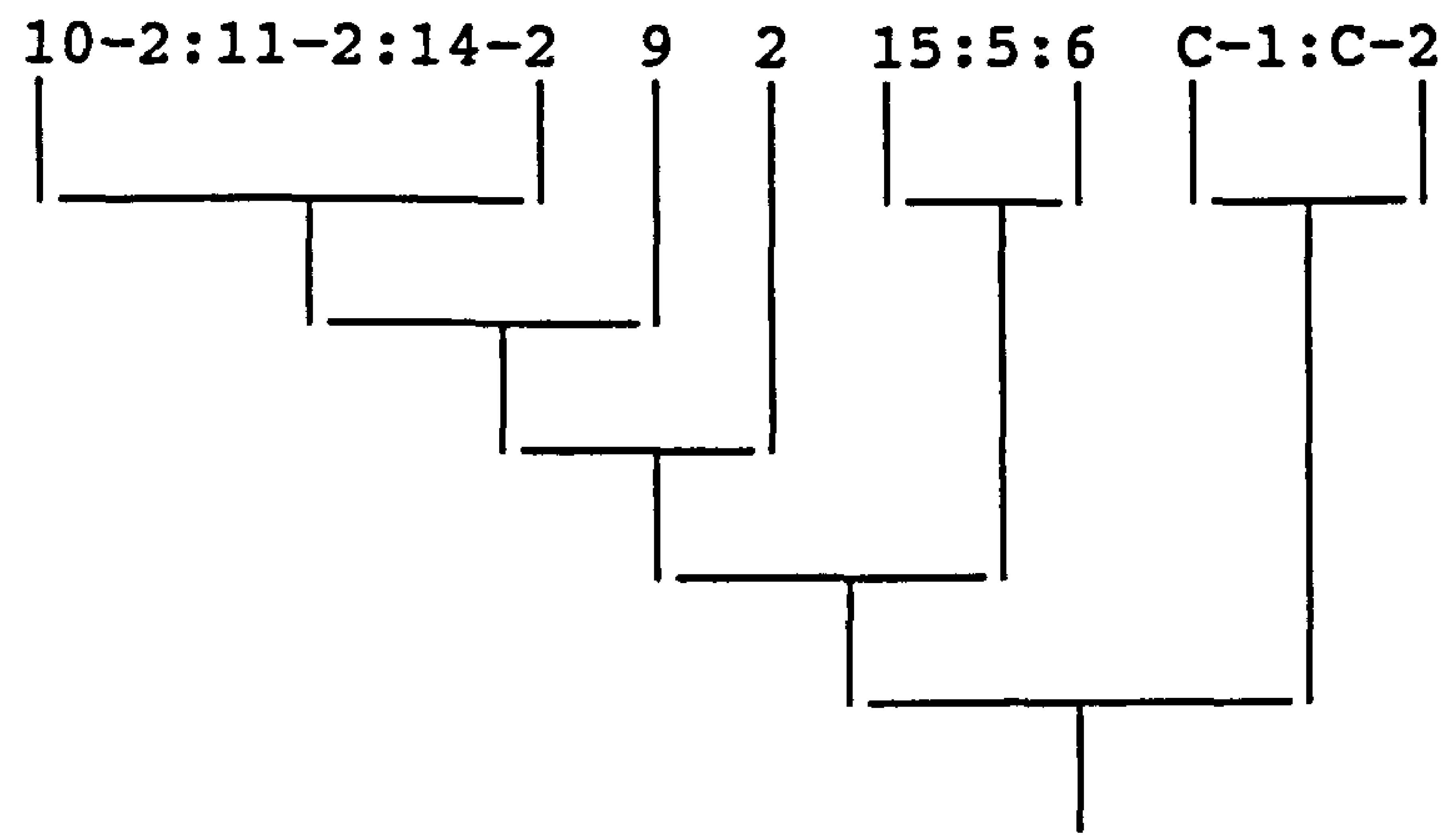
Subset 3, Chi-squared



Subset 3, corrected Chi-squared

No divisions performed

Subset 4, Chi-squared



Subset 4, corrected Chi-squared

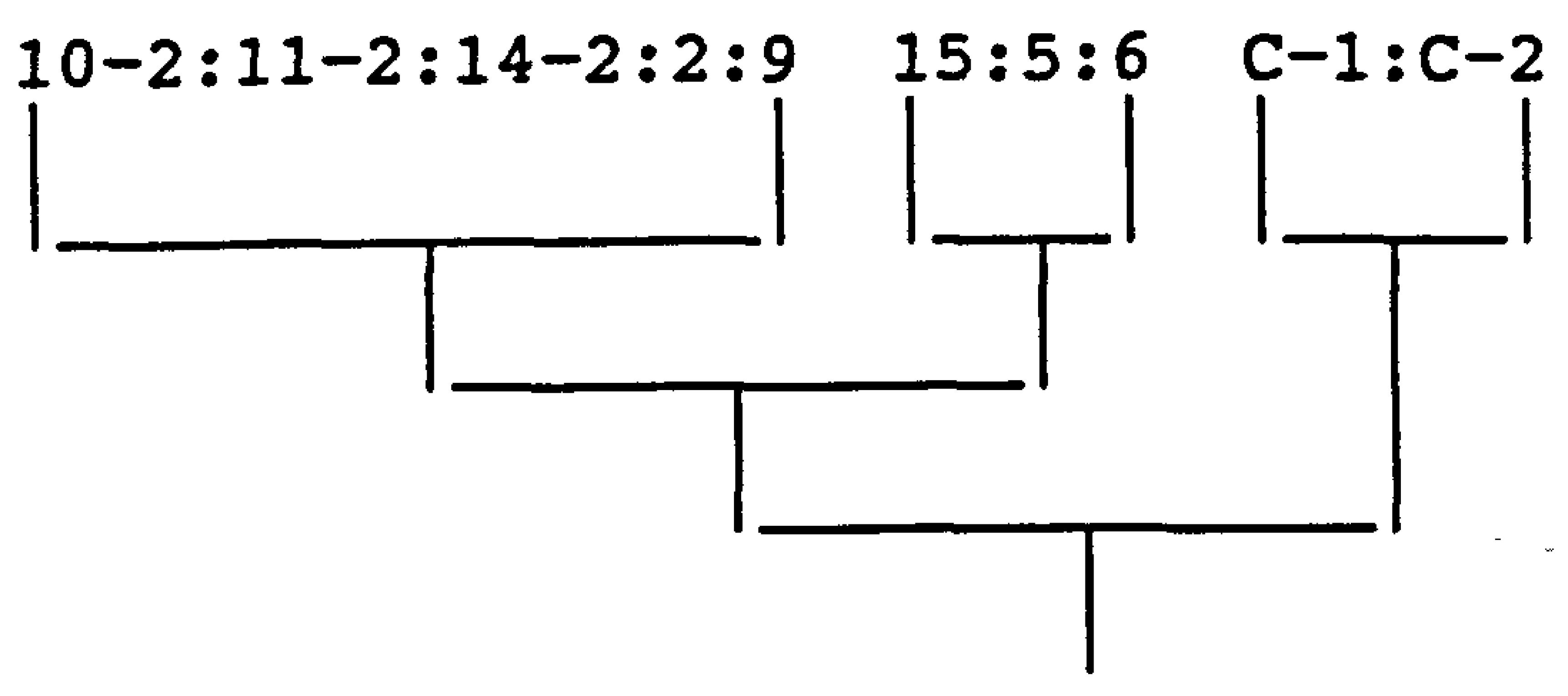
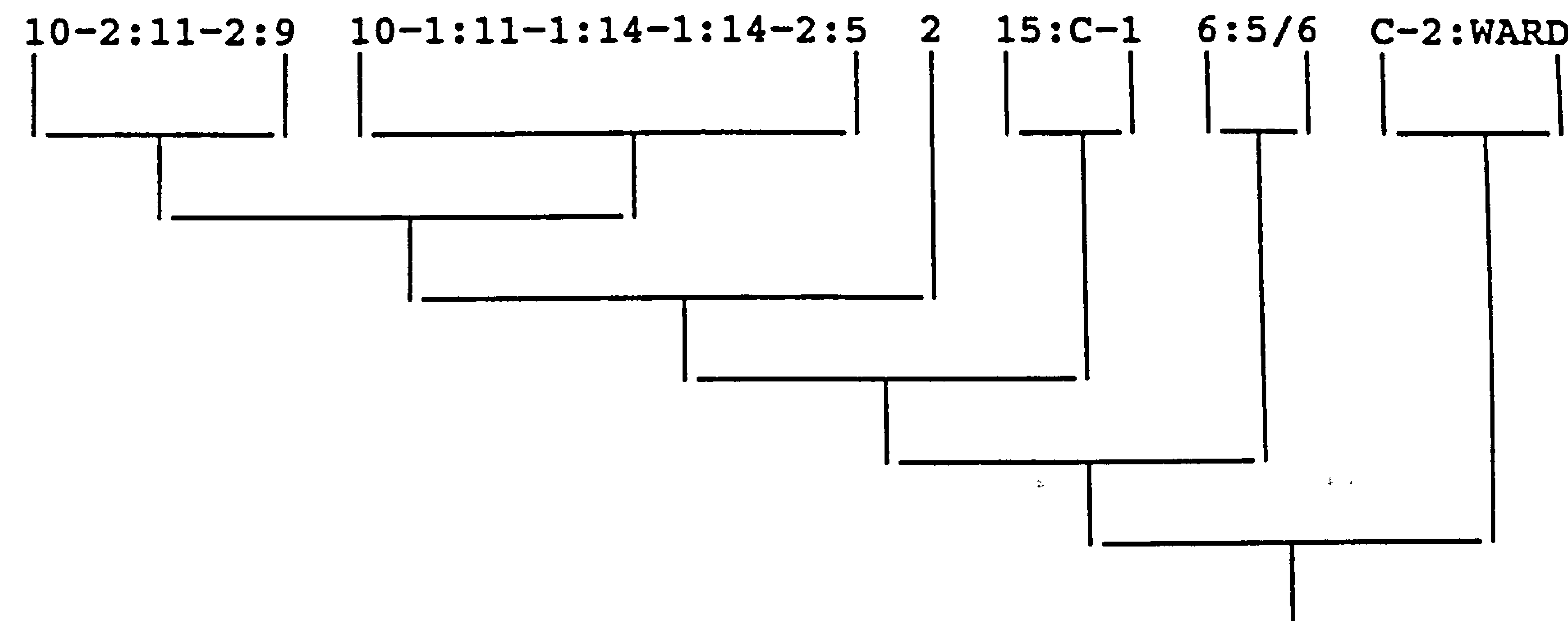


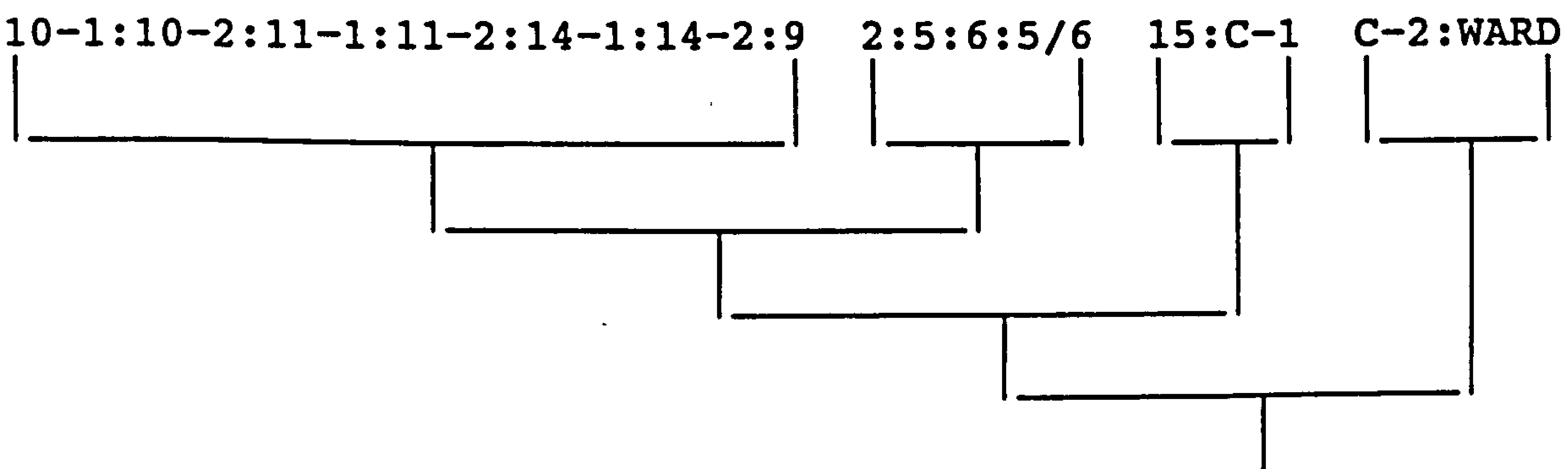
FIG.48

NASSOC Sampling Unit Associations:
Subsets 5 and 6 compared

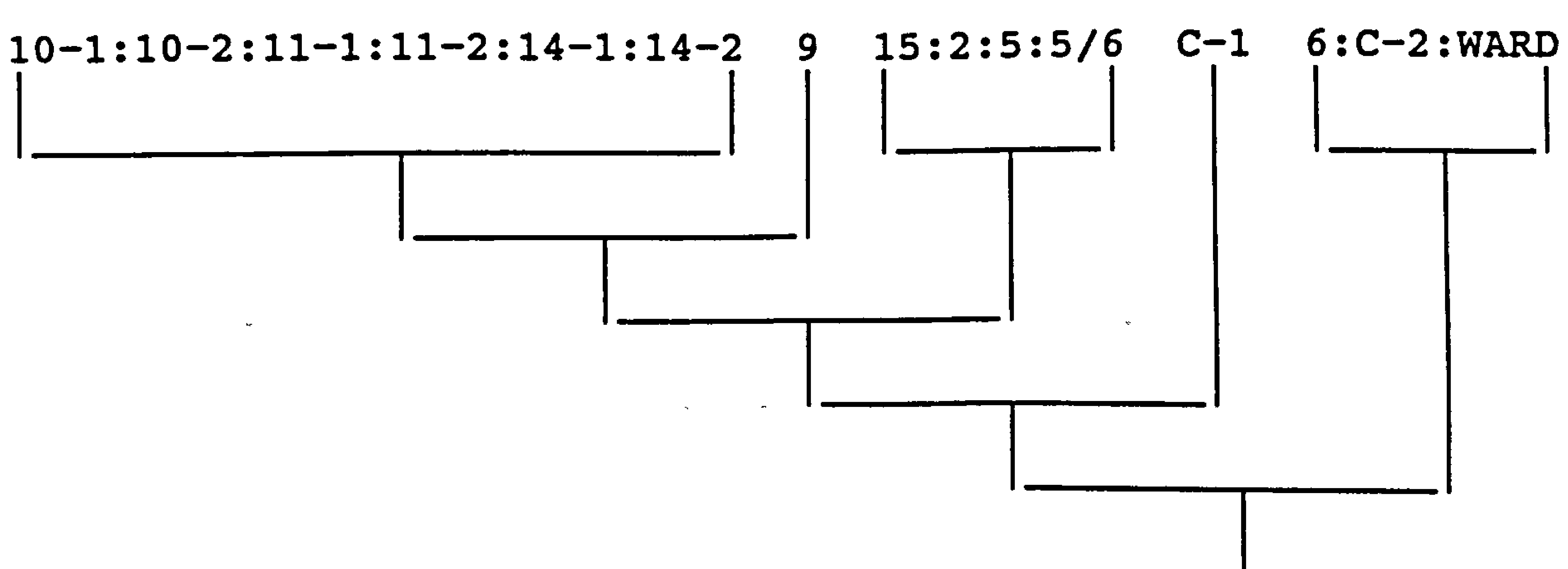
Subset 5, Chi-squared



Subset 5, corrected Chi-squared



Subset 6, Chi-squared

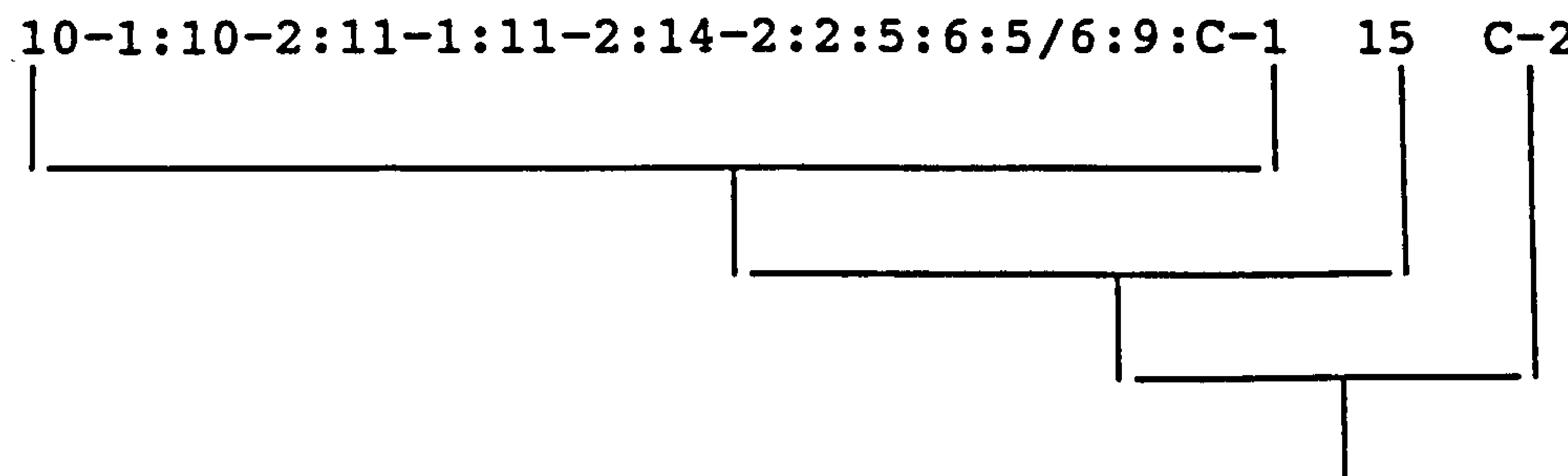


Subset 6, corrected Chi-squared
No divisions produced

FIG.49

NASSOC Sampling Unit Associations:
Subsets 7 and 8 compared

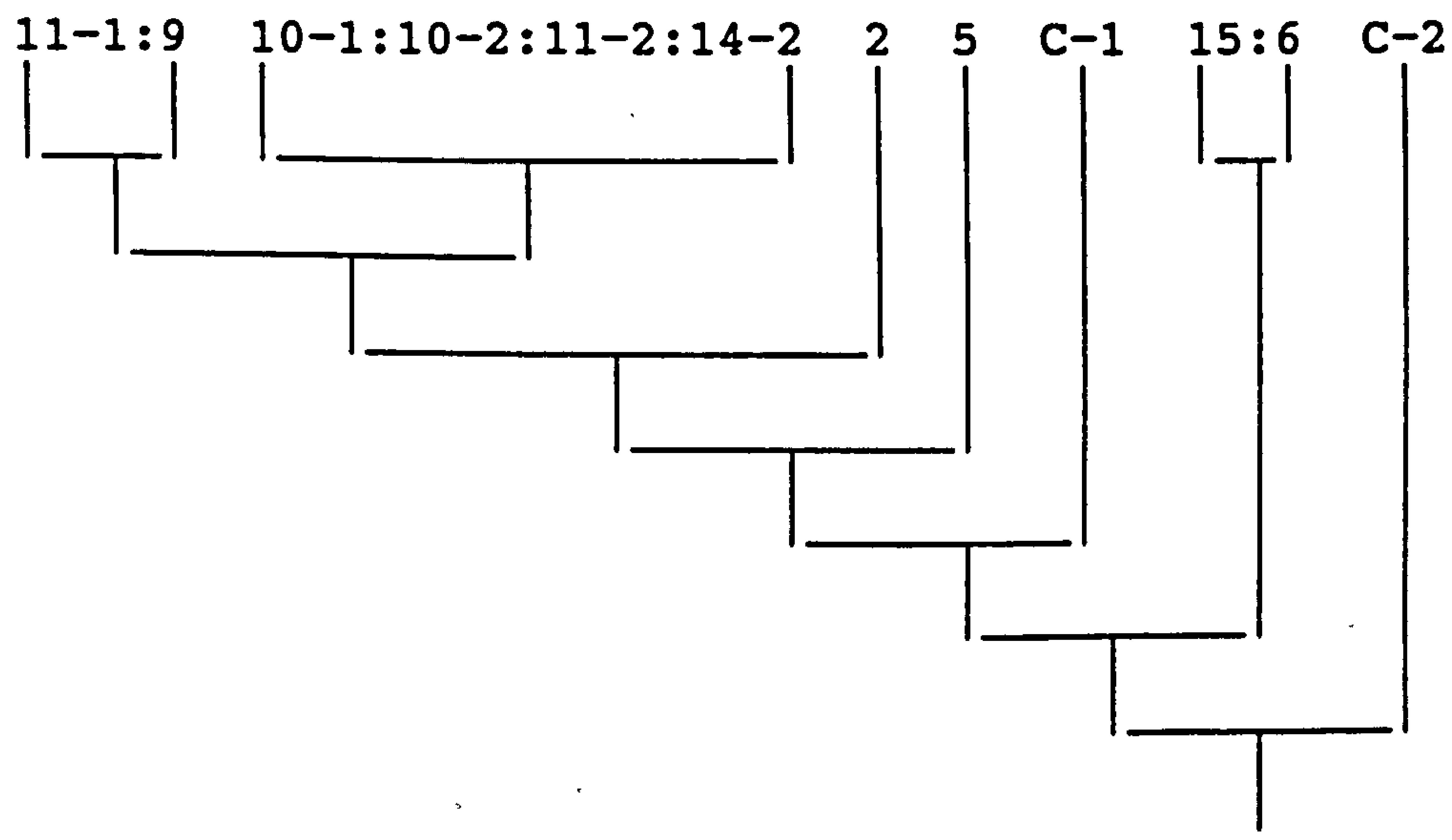
Subset 7, Chi-squared



Subset 7, corrected Chi-squared

No division by corrected Chi-squared

Subset 8, Chi-squared



Subset 8, corrected Chi-squared

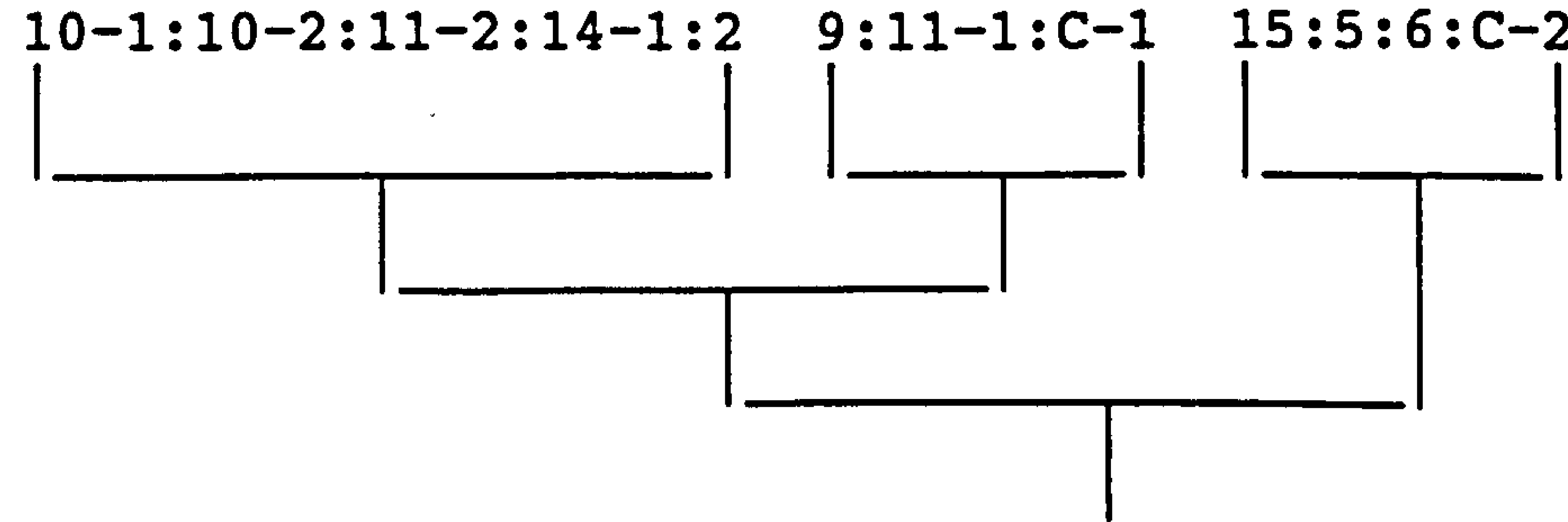
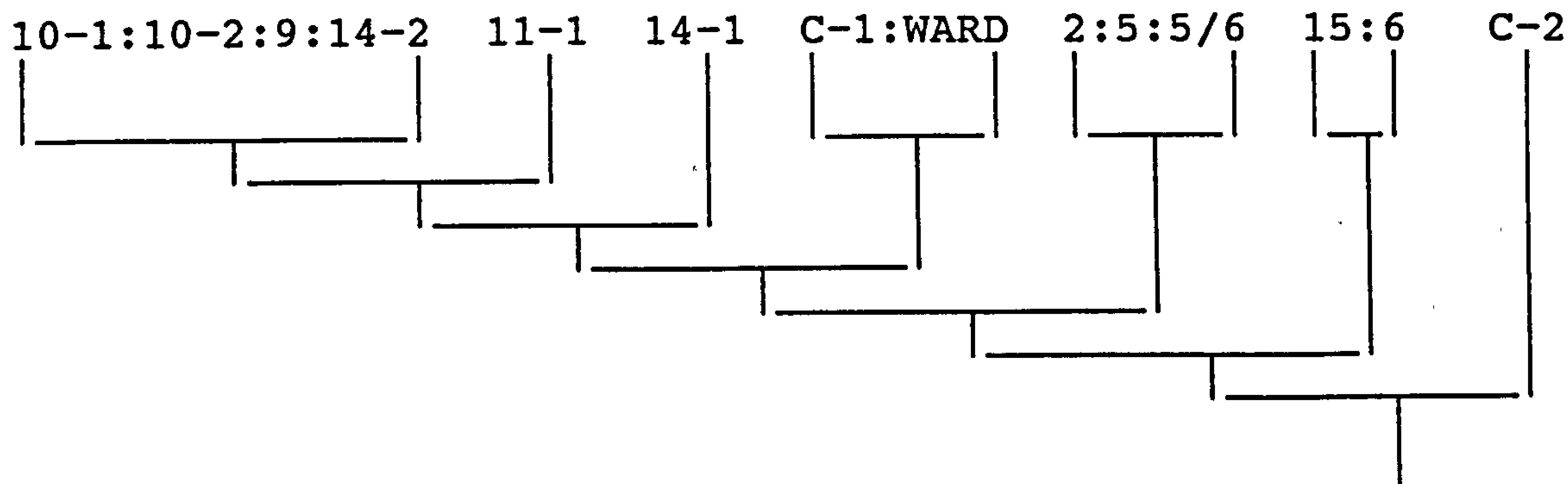


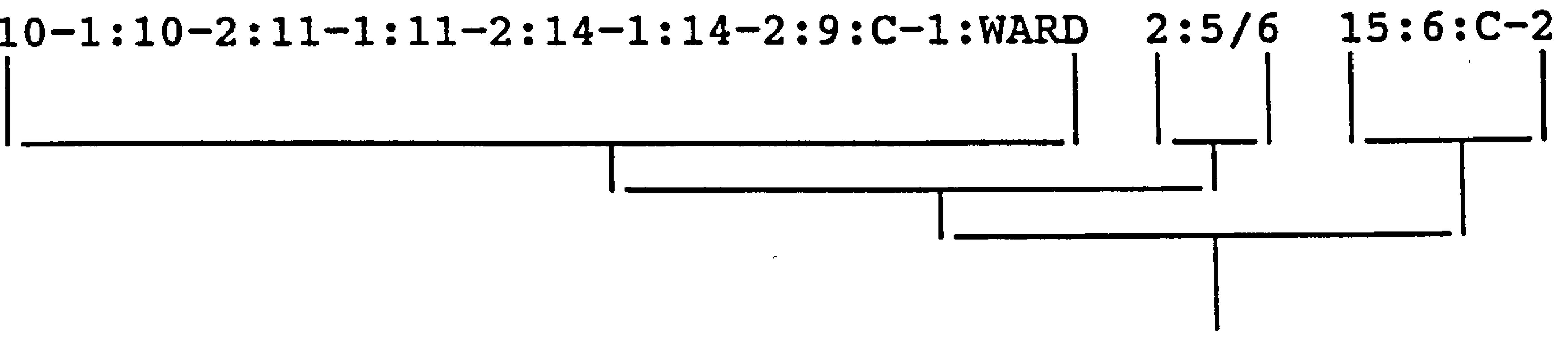
FIG.50

NASSOC Sampling Unit Associations:
Subsets 10 and 11 compared

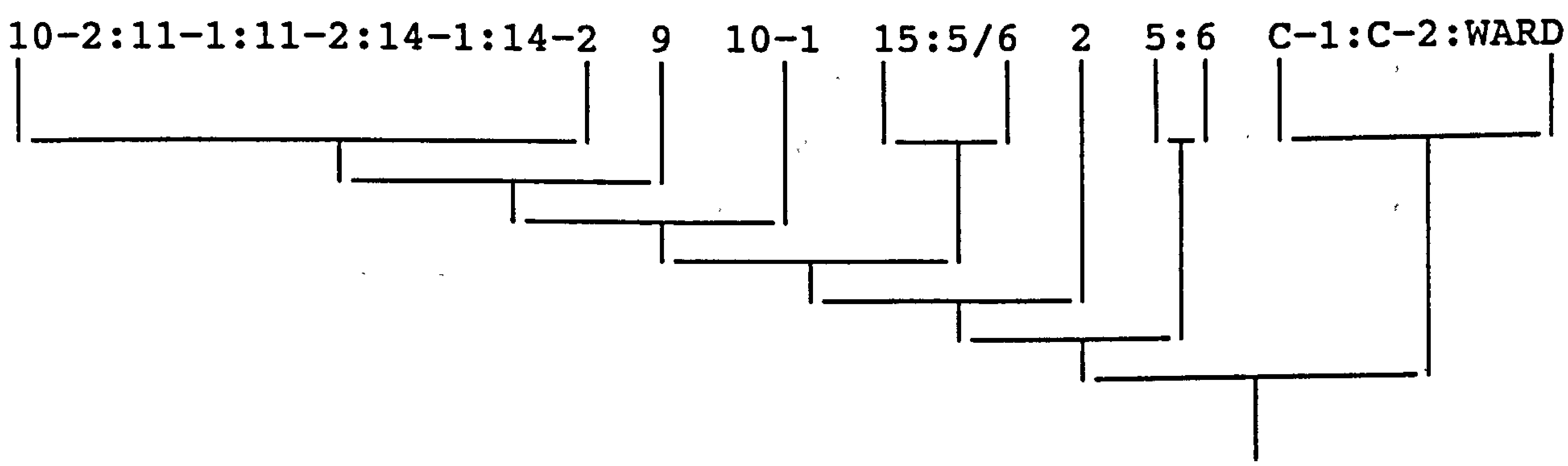
Subset 10, Chi-squared



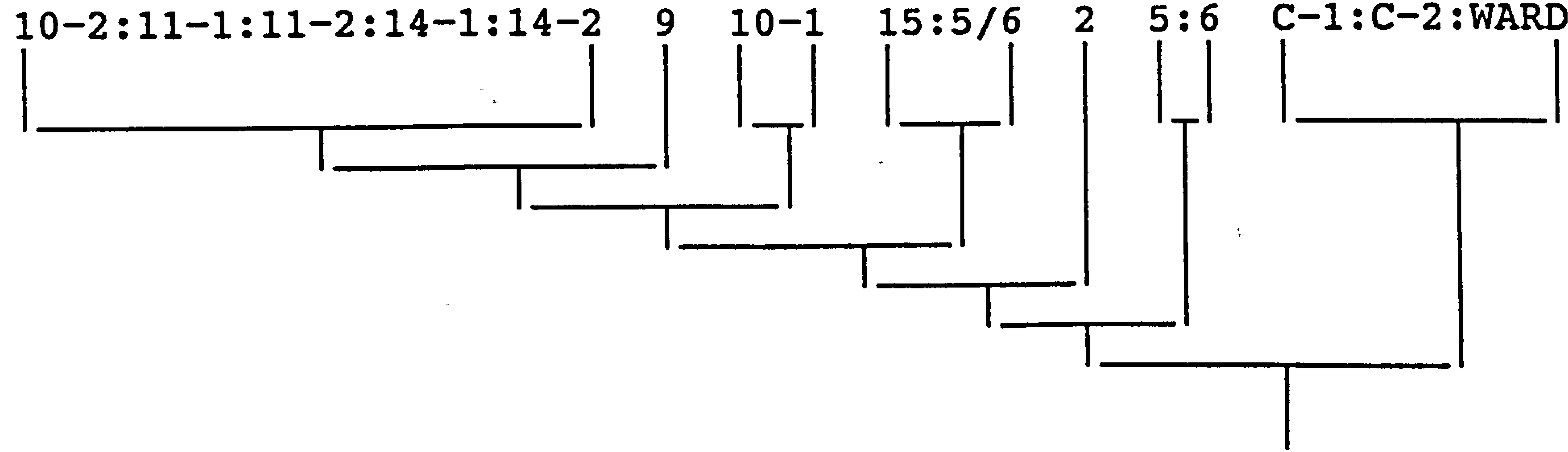
Subset 10, corrected Chi-squared



Subset 11, Chi-squared



Subset 11, corrected Chi-squared



corrected χ^2. It separates a group of sites which are characterised by having the highest mean pH values (Table 46) from the others. These sites are Ward, C-1, C-2, 15, 6, and in some analyses 5 also.

The second cycle divides the remaining sites into two groups, which may be interpreted as being a group of the lowest pH sites and an intermediate group, although the composition of the latter is somewhat variable. It should be noted that these designations are at this stage purely subjective, suggested by the observed distribution of mean pH shown in Table 46.

The data subsets used in Inverse Association Analysis (Section 4.4.2) were also subjected to NAA (Figs. 46-50). Here also the least acidic sites tend to be separated off earliest in the analysis but the results are much more variable. It is clear that the value of the analysis is greatly enhanced if the data set is based on a larger number of samples.

An interesting feature seen in Figs. 41 and 42 is that the burn 5 site is associated with the least acidic group in Matrix A, but with the most acidic group in Matrix B. If this is not simply a statistical aberration, it implies that burn 5 may be undergoing further acidification. In Figs. 43 and 44 however burn 5 is found to be associated with burns 2 and 5/6 which are intermediate in many of the analyses. It is also noteworthy that 14-1, 5/6 and Ward form one group in Matrix A, Figs 43 and 44, due to the paucity of samples. Thus insufficient or unevenly distributed data can bias the results.

4.6.4 Cluster Analysis

4.6.4.1 Choice of method

The cluster analysis may be carried out on several distance measures by several different strategies; the CLUSTER.BAS program includes 10 distance measures and 4 strategies including a flexible strategy in which the analyst chooses the value of β to be used in the computation. Thus up to approximately 100 different cluster analyses may be carried out on each data set. The results may be quite variable and it might be possible to choose an analysis to fit in with a number of different hypotheses. Some of the clustering methods may be favoured *a priori* on theoretical grounds (Ludwig and Reynolds, 1988).

Ludwig and Reynolds recommend in particular the use of Percent Dissimilarity and Chord distance as they give less weight to the higher abundance groups. Also, Relative Euclidean distances are preferable to Euclidean distance measures which may in some circumstances give spurious results (Ludwig and Reynolds, 1988). Since the data matrices are based on relative abundance measures rather than absolute numbers it may be questioned whether the use of Chord or P.D. is as advantageous in this case. The cluster analyses were carried out using Mean Absolute Distance (a Euclidean measure), P.D., Chord and Relative Euclidean Distance, by both the group-average strategy and the flexible strategy with β ranging from +0.25 to -0.5.

Pinel-Alloul *et al* (1990) utilized a β value of -0.25 in order to minimise chaining. A β value of -0.25 tends to be space conserving, i.e. not distorting the distances between S.U.s (Ludwig and Reynolds, 1988).

4.6.4.2 Results

Most procedures produce clusters in which the circumneutral sites (burn 15, Corrie (upper and lower) and Ward burn) are separated from the acidic sites (burns 14, 9, 10 and 11) with the remainder (burns 2, 5, 6, 5/6) forming an intermediate group whose members might in some procedures be linked more closely with one of the other groups. In some cluster sets, burns 14 and 11-2 are attached more closely to burns 2 and 5, the more acidic of the intermediate group.

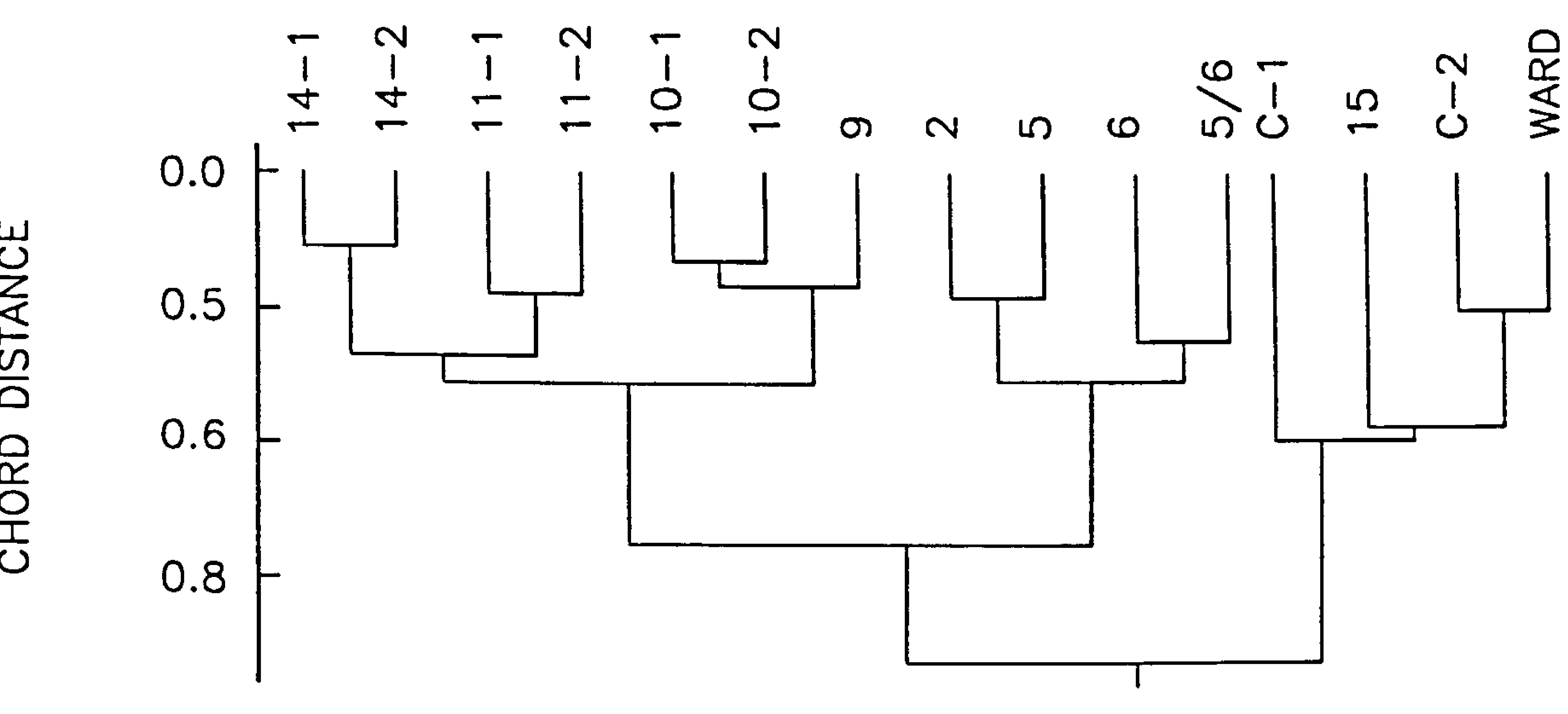
MAD frequently gives rise to chaining, in which clear groups are not formed but S.U.s are attached one at a time.

Intuitively an analysis might appear more valid if the upstream/downstream sites on the same burns are paired up early in the clustering. This is true in a large number of procedures for burns 14, 10 and, slightly less frequently, 11. It might not be expected for the Corrie burn as the upper Corrie site (C-1) is situated above the confluence of a tributary which though small is nonetheless the least acidic of 4 feeder streams. It is also found that burns 2 and 5 are frequently paired up early in the clustering.

The performance of the different clustering procedures on the different data matrices is compared in Table 47.

FIG.51:— CLUSTER ANALYSIS OF MATRIX B SPECIES DATA: FLEXIBLE STRATEGY WITH $\beta=-0.25$

Pooled P/A data



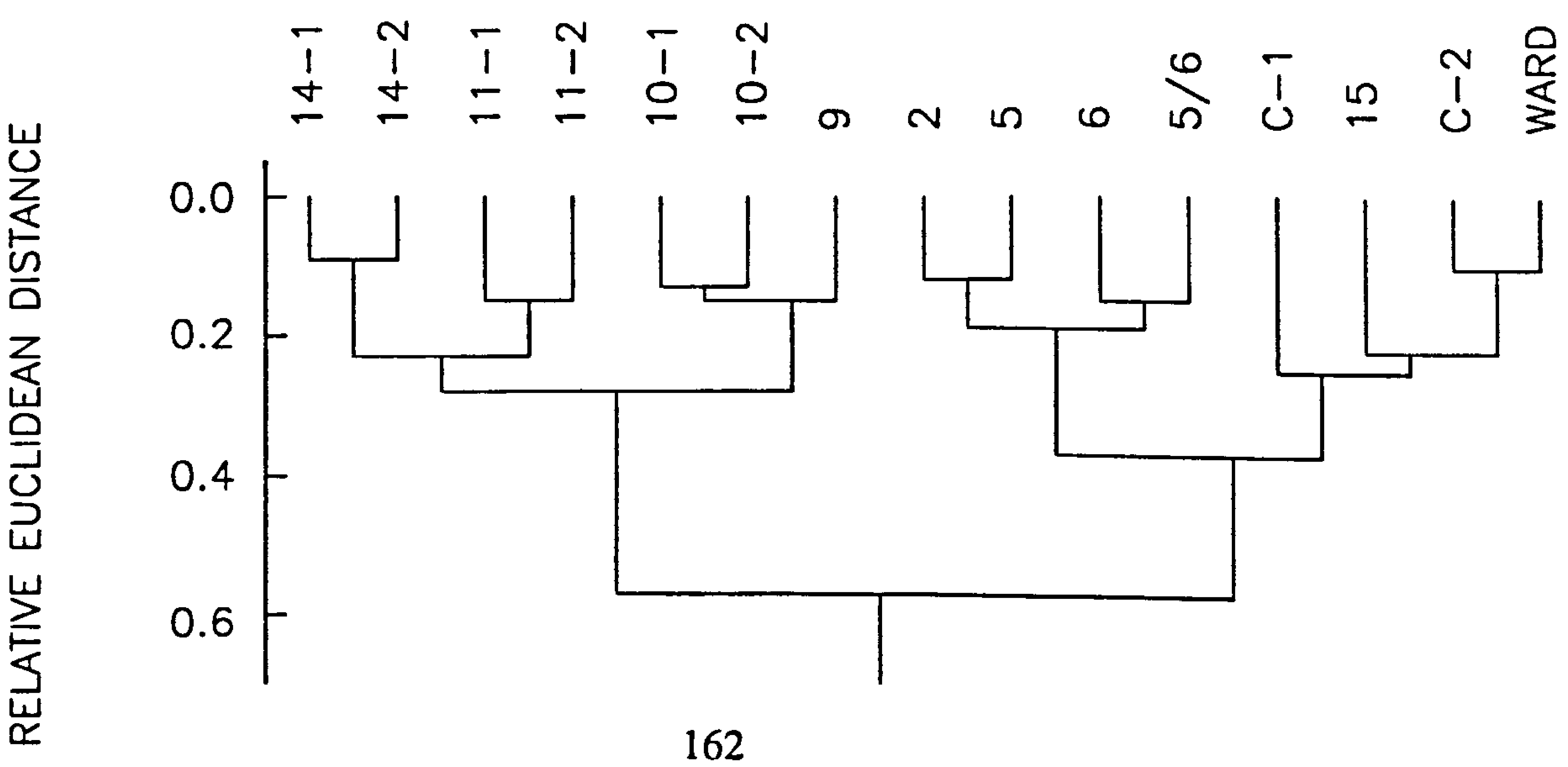
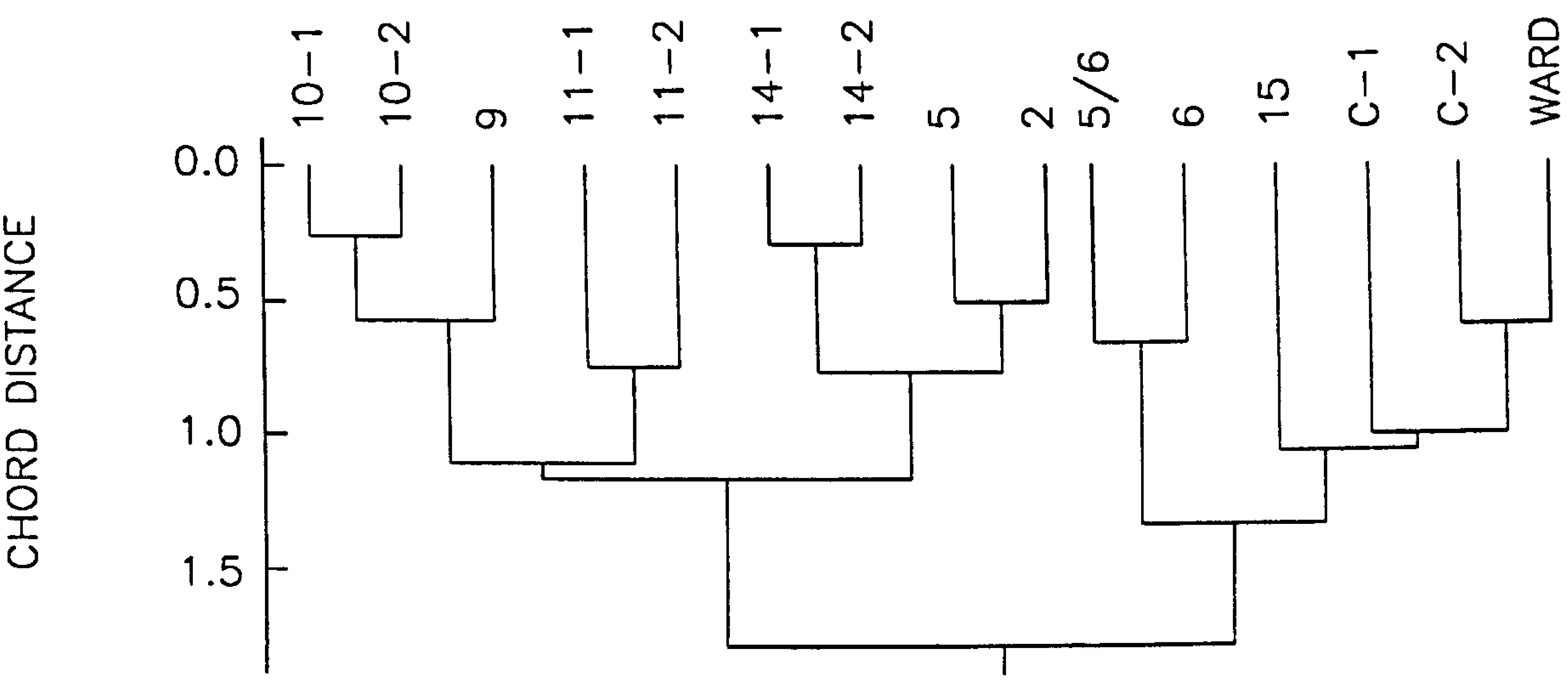


FIG.52:- CLUSTER ANALYSIS OF MATRIX B SPECIES DATA: FLEXIBLE STRATEGY WITH $\beta=-0.25$

Abundance data



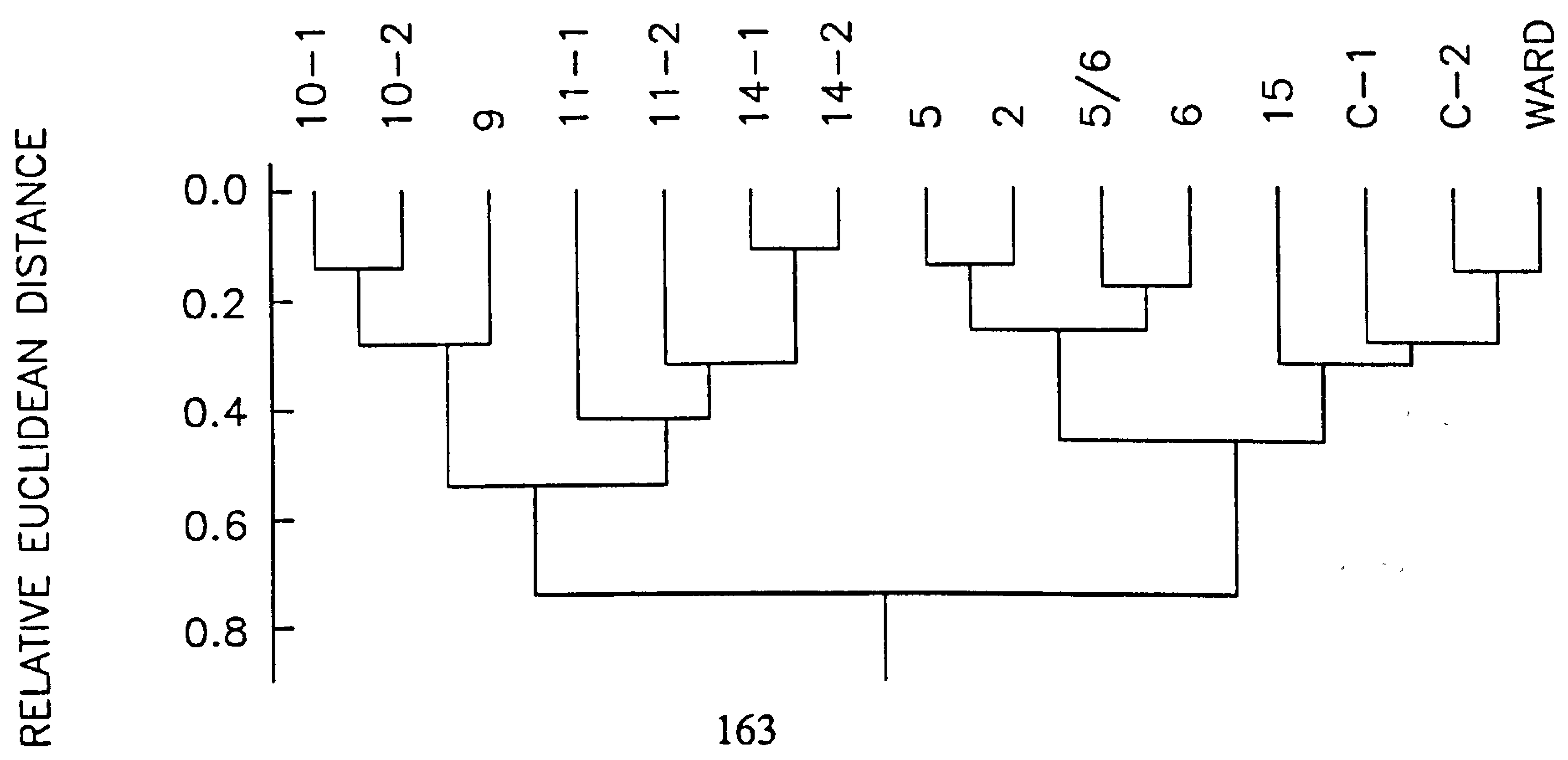


TABLE 47
CLUSTER ANALYSIS RESULTS

Dist-ance Meas-ure	Strategy	Matrix A						Matrix B						Matrix C					
		abund.			p/a			abund			p/a			abund			p/a		
		p	t	c	p	t	c	p	t	c	p	t	c	p	t	c	p	t	c
		2	7	12	2	7	12	3	7	13	3	7	13	3	7	13	3	7	13 = Max
MAD	G-A	1	3	8	0	4	6	2	4	7	1	4	6	2	5	5	2	4	7
	FLEX:																		
	=0.25	1	5	4	0	7	0	3	7	1	1	7	1	3	7	1	3	5	5
	0.125	1	4	6	0	6	2	3	6	3	1	6	3	3	7	1	2	5	5
	0	1	3	8	0	4	6	3	4	7	1	5	5	2	4	7	2	4	7
	-0.125	1	3	8	0	4	6	3	6	3	1	5	5	2	6	3	2	5	5
	-0.25	1	3	8	0	4	6	3	5	5	1	6	3	2	6	3	2	6	3
	-0.50	1	3	8	0	5	4	3	6	3	1	6	3	2	6	3	2	6	3
P.D.	G-A	0	3	8	0	3	7	2	5	5	1	5	5	2	6	3	3	5	5
	FLEX:																		
	=0.25	0	7	0	0	6	2	2	7	1	1	6	3	2	7	1	3	7	1
	0.125	0	5	4	0	5	4	2	6	3	1	6	3	2	6	3	3	6	3
	0	0	3	8	0	5	4	2	5	5	1	5	5	2	6	3	3	5	5
	-0.125	0	4	6	0	5	4	2	5	5	1	5	5	2	6	3	3	5	5
	-0.25	0	5	4	0	5	4	2	5	5	1	5	5	2	6	3	3	5	5
	-0.50	0	6	2	0	5	4	3	7	1	1	6	3	2	7	3	3	5	5
RED	G-A	1	5	4	1	3	8	3	6	3	3	6	3	2	5	5	3	6	3
	FLEX:																		
	=0.25	1	6	2	1	6	2	3	7	1	3	7	1	2	7	1	3	7	1
	0.125	1	6	2	1	5	3	3	6	3	3	6	3	2	6	3	3	7	1
	0	1	5	4	1	3	8	3	6	3	3	6	3	2	5	5	3	6	3
	-0.125	1	5	4	1	3	8	2	5	5	3	6	3	2	5	5	3	6	3
	-0.25	1	5	4	1	4	6	2	5	5	3	6	3	2	5	5	3	6	3
	-0.50	1	5	4	1	4	6	3	6	3	3	6	3	2	6	3	3	6	3
CHORD	G-A	1	4	6	1	3	8	2	5	5	3	6	3	2	5	5	3	6	3
	FLEX:																		
	=0.25	1	7	0	1	6	2	3	7	1	3	7	1	3	7	1	3	7	1
	0.125	1	4	6	1	6	2	3	7	1	3	7	1	2	6	3	3	7	1
	0	1	7	0	1	3	8	3	6	3	3	6	3	2	5	5	3	6	3
	-0.125	1	4	6	1	5	5	3	6	3	3	6	3	2	5	5	3	6	3
	-0.25	1	6	2	1	5	5	3	6	3	3	7	1	2	5	5	3	6	3
	-0.50	1	6	2	1	5	5	3	7	1	3	7	1	2	5	5	3	6	3

DISTANCE MEASURES:
MEAN ABSOLUTE DISTANCE
PERCENT DISSIMILARITY
RELATIVE EUCLIDEAN DISTANCE
CHORD DISTANCE

CLUSTERING STRATEGIES:
- GROUP AVERAGE
- FLEXIBLE
(choice of β value)

p = number of pairs formed between upstream and downstream sites
t = total pairs
c = number of sites linked singly (chaining)

The first value 'p' shows the number of pairings of upstream and downstream sites, e.g. 14-1 with 14-2. There are two such possible pairings in Matrix A and three in B and C. Thus the higher the value in this column, the closer is the result to the expectation that such sites should be similar.

The second value 't' shows the total number of pairs formed in the dendrogram. The third value 'c' shows the number of sites which are attached by 'chaining'. Thus t and c show the relative contribution of pairing and chaining.

The chaining phenomenon may be useful in that it shows a relative order of the sites, which is not apparent from highly paired dendrograms, since a cluster may be rotated about the common stem. This information may then be used to order the sites in a dendrogram involving pairings. However different clustering procedures may result in mutually exclusive interpretations of the interrelationship between sites.

The order of the sites shown in the cluster dendrograms (Figs. 51, 52) has been drawn up using evidence from chaining.

For a comparison of the relative virtues of different approaches, Matrix B data (the second half of the time series) provides the most complete data set, as all sites were sampled during this period (not all on every occasion however). The data may be utilized in two ways (a) as a matrix based on the mean relative abundances of the taxa or (b) as a matrix obtained by summing the presence/absence scores on each sampling occasion (giving a maximum score of 18 for any species in any site).

The Presence/Absence (p/a) data are found to give more consistent pairing of upstream-downstream sites than the abundance data. Figs. 51 and 52 shows a comparison of both data sets clustered by the Flexible strategy, with $\beta= -0.25$, using Chord distance and Relative Euclidean Distance.

This may be taken as some evidence for the superiority of the presence-absence form of the data. However the differences are very slight and it might not be correct to expect a very close pairing between 11-1 and 11-2 as the former is in a very open site with relatively slow-moving water and a heavy growth of mosses and liverworts, while the latter is heavily shaded by trees and lies downstream of a substantial waterfall, factors which may result in some floral differences.

Therefore on the whole the results obtained using the pooled P/A data tend to support those obtained with relative abundance data, dividing the sites into a circumneutral set and an acidified set.

Intermediate between these are the sites on burns 2, 5 and 6 which are attached to the acidic set in the Chord Distance clusterings, and to the circumneutral set in RED clustering.

These are burns which are at risk of acidification, being originally base-poor, moorland streams with low humic content. Burn 2 is still in this condition, with a very low percentage of forest cover (about 100 m of bankside conifers), while the catchments of burns 5 and 6 are unforested in their upper reaches, but with young coniferous plantation surrounding the sites and extending about half a mile upstream.

With the growth of the trees approaching canopy closure, the drainage water may be expected to acidify further (Nilsson *et al*, 1982; Nilsson, 1983) depending on the nature of the precipitation. Burns 2 and 6 currently still support the presence of fish, while burn 5 does not.

The composition of the algal flora therefore can be used to group the sites as shown by correlations between sites in respect of their species composition (Species Covariation), by Species Association and Normal Association Analysis based on presence-absence data, and by Cluster Analysis based on both abundance and pooled P/A data, and this grouping is related to the acidification status of the sites.

4.7 SPECIES-ENVIRONMENT RELATIONSHIPS

4.7.1 Preliminary Considerations: choice of method

The type of analysis to be pursued depends on whether the relationship between species and environmental variables is best described by a linear or unimodel model. If the environmental gradient is short, a linear model may be appropriate (Ter Braak and Prentice, 1988).

The length of the environmental gradient formed by each of the variables measured is shown in Table 48. The majority exceed 3 S.D. and none is less than 2. The non-linear methods carried out by CANOCO are relevant if the gradient is more than 3 S.D. in length and should not be used if it is less than 1.5 (Ter Braak and Prentice, 1988).

To determine whether a linear model is appropriate for particular species, exploratory analyses were carried out. The Pearson correlation coefficient 'r' was calculated between individual species and each environmental variable in turn (Table 49). The appropriate significance level depends on the number of correlations in the set. With 49 species and 16 variables, there are 784 correlations, so a strong correlation might be obtained purely by chance at a significance level less than 0.1%. At this level, the value of r which is significant depends on the number of data points on which each correlation is calculated: e.g. for pH a correlation is significant if it exceeds 0.165. Since the number of data points differ depending on the number of measurements made of each variable, the 0.1% significance value of r for each is included in the table.

The possibility that transformation of the environmental data might yield larger correlation coefficients was considered. Inspection of the data points in plots of species abundance versus environmental variables suggested that a Log_{10} or square root transformation might be appropriate.

The effect of Log_{10} and square-root transformation of environmental variables was initially investigated through correlation analysis of total species number per sample with environmental variables (Table 50) as a larger number of data points is available than with individual species.

Higher correlations are found with untransformed pH, alkalinity, Ca^{2+}, Mg^{2+}, SiO_2, and with Log_{10} transformed PO_4, Al-TM, Al-NL and Al-L.

TABLE 48
LENGTH OF ENVIRONMENTAL GRADIENTS, MATRIX C
ENVIRONMENTAL GRADIENT = RANGE/S.D.

Variable	Range	S.D.	Gradient length (S.D.units)
pH inst.	2.3	0.775	2.97
pH comp.	2.3	0.806	2.85
'FLOW'	0.29	0.093	3.10
'DEPTH'	0.096	0.033	2.87
TEMP	7.3	1.829	3.99
A_{400}	0.09	0.028	3.21
A_{350}	0.20	0.056	3.57
A_{250} inst.	0.59	0.187	3.16
A_{250} comp.	0.35	0.128	2.73
PAR	58	17.5	3.31
TON	21.1	6.49	3.25
PO_4	39	9.80	3.98
Al-TM	179.5	61.6	2.91
Al-NL	62.34	27.45	2.27
Al-L	112.48	37.17	3.03
ALKALIN	161	47.3	3.40
Ca^{2+}	135.1	38.9	3.47
Mg^{2+}	111.9	31.12	3.60
SiO_2	2128	577	3.69
H^+ inst.	42.14	17.08	2.46
H^+ comp.	40.02	15.36	2.61

Range and S.D. from mean data for each site.
The environmental gradient is a standardised expression of the spread of values of a variable encountered in the field.

TABLE 49

CORRELATIONS BETWEEN RELATIVE SPECIES ABUNDANCE AND ENVIRONMENTAL VARIABLES IN EACH SAMPLE

	SPECIES	pH	'FLOW'	'DEPTH'	A.400	A.350	A.250	PAR	TON	PO4	Al-TM	Al-NL	Al-LAB	ALKALIN	Ca	Mg	SiO2
1	Oscillatoria.1um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Oscillatoria.2um.	0.210	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Oscillatoria.5um.	0.392	-	-	-	-	-	-	0.206	-	-0.276	-0.227	-0.242	0.548	0.599	0.556	0.357
4	Oscillatoria.8um.	0.381	-	-	-	-	-	-	-	-	-0.281	-0.258	-0.238	0.663	0.653	0.614	0.386
5	Oscillatoria.11um	0.380	-	-	-	-	-	-	0.281	-	-0.294	-0.262	-0.249	0.674	0.701	0.678	0.416
6	Oscillatoria.14um	0.207	-	-	-	-	-	-	-	-	-0.121	-	-	0.381	0.359	0.348	-
7	Phormidium.3um.	0.192	-	-	-	0.304	-	-	-	-	-	-	-	-	-	-	-
8	Phormidium.5um.	0.526	-	-	-	-	-	-	-	-	-0.403	-0.341	-0.360	0.614	0.602	0.525	0.435
9	Phormidium_8um.	0.196	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Phormidium_13um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Tolypothrix	0.287	0.399	-	-0.179	-	-0.191	-	-	-	-0.387	-0.350	-0.335	-	-	-	-
12	Toly.epiphyte	-	0.327	-	-	-0.292	-	-	-	-	-0.246	-0.221	-0.214	-	-	-	-
13	Stigonema	-	-	-	-	-	-	-	-	-	-0.200	-	-	-	-	-	-
14	Scytonema	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Batrachospermum	0.294	0.227	-	-0.175	-	-0.179	-	-	-	-0.360	-0.325	-0.313	0.267	0.212	-	-
16	Lemanea	0.465	-	-	-	-	-	-	-	-	-0.355	-0.334	-0.294	0.544	0.487	0.435	0.483
17	Audouinella	0.450	-	-	-	-	-	-	0.298	-	-0.326	-0.270	-0.297	0.745	0.762	0.750	0.556
18	Audouinella(green	0.193	-	-	-	-	-	-	-	-	-	-	-	0.220	-	-	-
19	Draparnaldia	0.367	-	-	-	-	-0.166	-	-	-	-0.407	-0.384	-0.325	0.709	0.627	0.510	0.365
20	Stigeoclonium_5um	-0.277	-0.244	-0.225	-	-	-	-	-	-	-	-	-	-0.221	-	-	-
21	Stigeoclonium_8um	0.392	-	-	-	-	-	0.331	-	-	-0.245	-0.221	-0.212	-	-	-	0.383
22	Oedogonium_6um.	0.321	0.331	-	-0.182	-	-0.190	0.340	-	-	-0.493	-0.394	-0.473	0.222	-	-	-
23	Oedogonium_14um.	0.277	-	-	-	-	-	-	-	-	-0.229	-	-0.223	0.217	0.240	-	-
24	Oedogonium_30um.	0.399	-	-	-	0.292	0.174	-	-	-	-0.211	-	-0.211	0.528	0.552	0.552	0.513
25	Spirogyra_20um.	0.248	-	-	-	-	-	-	-	-	-	-	-	0.380	0.363	0.256	-
26	Spirogyra_32um.	0.239	-	-	-	-	-	-	-	-	-	-	-	0.485	0.460	0.413	-
27	Zygnema_20um.	0.261	0.404	-	-	-	-0.165	0.325	-	-	-0.384	-0.323	-0.355	-	-	-	-
28	Zygnema_28um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	Mougeotia_5um.	-	-	-0.224	-	-	-	-	-	-	-	-	-	0.255	0.284	0.228	-
30	Mougeotia_11um.	0.218	-	-0.350	-	-	-	-	-0.226	-	-0.390	-0.374	-0.325	-	-	-	-
31	Mougeotia_16um.	-	-	-0.193	0.184	-	-	-	-	-	-	-	-	-	-	-	-
32	Mougeotia_20um.	0.274	-	-	-	-	-	-	-	-	-0.289	-0.263	-0.249	0.245	0.241	-	-
33	Mougeotia_25um.	0.321	-	-	-	-	-	-	-	-	-0.313	-0.288	-0.266	0.576	0.503	0.464	-
34	Microthamnion	-	-	-	-	-	-	-	-	0.448	-	-	-	-	-	-	-
35	Microspora_8um.	-	-	-0.247	-	-	-	-	-0.269	-	-	-	-	-	-	-	-
36	Microspora_11um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	Microspora_14um.	-0.209	-0.220	-	0.364	0.303	0.355	-	-	-	-	-0.225	-	-	-	-	-
38	Microspora_22um.	0.310	-	-	-	-	-	-	-	-	-	-	-	0.487	0.472	0.452	0.346
39	Geminella_8um.	0.193	-	-	-	-	-	-	-	-	-0.282	-0.311	-0.211	0.218	0.200	-	-
40	Geminella_11um.	0.250	-	-	-	-	-	0.347	-	-	-0.284	-0.218	-0.274	0.299	0.307	0.234	-
41	Hormidium_5um.	-0.304	-	-	-	-	-	-	-	-	-	-	-	-0.302	-0.377	-0.370	-0.399
42	Hormidium_8um.	-0.234	-	0.371	-	-	-	-	-	-	-	-	-	-	-	-	-0.313
43	Ulothrix_8um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	Ulothrix_11um.	-	-	0.236	-	-	-	-	-	0.480	-	-	-	-	-	-	-
45	Ulo./scaly_11um.	-	-	0.217	-	-	-	-	-	-	-	-	-	-	-	-	-
46	Ulothrix_14um.	0.203	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	Ulothrix_17um.	0.214	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	Ulothrix_28um.	0.237	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	Ulothrix_36um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	SIGNIFICANT @ 0.1% =	0.165	0.193	0.193	0.165	0.273	0.165	0.312	0.199	0.312	0.197	0.198	0.205	0.299	0.196	0.196	0.298

TABLE 50
CORRELATION ANALYSIS OF TOTAL SPECIES NUMBER PER SAMPLE WITH UNTRANSFORMED AND LOG_{10} ENVIRONMENTAL VARIABLES

VARIABLE	PEARSON'S r		value of r (+/-) significant at 0.1%
	untransformed	Log_{10} transformed	
pH	0.633 #	-	0.164
'DEPTH'	-0.253	-0.253	0.193
A_{400}	-0.105	-0.146	0.165
A_{350}	-0.131	-0.206	0.270
A_{250}	-0.113	-0.154	0.164
TON	-0.038	-0.094	0.198
PO_4	-0.094	-0.320 #	0.312
Al - T.M.	-0.587	-0.661 #	0.196
Al - N.L.	-0.527	-0.584 #	0.198
Al - L	- 0.509	-0.657 #	0.205
Alkalinity	0.619 #	0.528	0.299
Ca^{++}	0.589 #	0.525	0.196
Mg^{++}	0.418 #	0.313	0.196
SiO_2	0.446	0.205	0.298
PAR	0.260	0.251	0.312

values exceeding the 0.1% significance level; the higher value of the pair is indicated

TABLE 51

CORRELATIONS BETWEEN RELATIVE SPECIES ABUNDANCE AND LOGTEN TRANSFORMED ENVIRONMENTAL VARIABLES IN EACH SAMPLE

	SPECIES	L.DEPTH	L.A400	L.A350	L.A250	L.PAR	L.TON	L.PO4	L.Al-TM	L.AlNL	L.Al-L	L.ALK	L.Ca	L.Mg	L.SiO2
1	Oscillatoria.1um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Oscillatoria.2um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Oscillatoria.5um.	-	-	-	-	-	0.204	-	-0.320	-0.251	-0.321	0.427	0.442	0.424	-
4	Oscillatoria.8um.	-	-	-	-0.168	-	-	-	-0.388	-0.397	-0.322	0.496	0.473	0.449	-
5	Oscillatoria.11um	-	-	-	-	-	0.255	-	-0.375	-0.335	-0.334	0.529	0.518	0.508	-
6	Oscillatoria.14um	-	-	-	-	-	-	-	-	-	-	-	0.244	0.247	-
7	Phormidium.3um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Phormidium.5um.	-	-	-	-0.173	-	-	-	-0.472	-0.416	-0.452	0.515	0.489	0.414	-
9	Phormidium_8um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Phormidium_13um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Tolypothrix	-	-0.262	-0.373	-0.268	-	-	-0.382	-0.403	-0.346	-0.434	-	-	-	-
12	Toly.epiphyte	-	-0.210	-0.281	-0.218	-	-	-	-0.252	-0.200	-0.281	-	-	-	-
13	Stigonema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	Scytonema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Batrachospermum	-	-0.211	-0.348	-0.199	-	-	-	-0.413	-0.385	-0.382	-	-	-	-
16	Lemanea	-	-	-0.232	-	-	-	-	-0.420	-0.355	-0.404	0.423	0.381	0.331	-
17	Audouinella	-	-	-	-	-	0.215	-	-0.379	-0.303	-0.351	0.597	0.576	0.572	-
18	Audouinella(green	-	-	-	-	-	-	-	-0.208	-	-	-	-	-	-
19	Draparnaldia	-	-0.175	-	-0.193	-	-	-	-0.513	-0.475	-0.464	0.559	0.481	0.377	-
20	Stigeoclonium_5um	-0.218	-	-	-	-	-	0.362	-	-	-	-	-	-	-
21	Stigeoclonium_8um	-	-	-	-	-	-	-	-0.249	-0.218	-0.241	-	-	-	-
22	Oedogonium_6um.	-	-0.178	-0.297	-0.201	0.357	-	-	-0.500	-0.412	-0.564	-	-	-	-
23	Oedogonium_14um.	-	-	-0.282	-0.190	-	-	-	-0.233	-	-0.294	-	0.211	-	-
24	Oedogonium_30um.	-	-	-	0.176	-	-	-	-0.214	-	-0.266	0.426	0.421	0.429	-
25	Spirogyra_20um.	-	-	-	-	-	-	-	-0.225	-0.231	-0.222	0.260	0.264	-	-
26	Spirogyra_32um.	-	-	-	-	-	-	-	-0.238	-0.224	-0.211	0.333	0.310	0.298	-
27	Zygnema_20um.	-	-0.184	-0.262	-0.195	0.324	-	-	-0.380	-0.303	-0.470	-	-	-	-
28	Zygnema_28um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	Mougeotia_5um.	-0.252	-	-	-	-	-	-	-0.212	-0.285	-0.205	-	0.275	0.209	-
30	Mougeotia_11um.	-0.385	-0.170	-0.292	-0.165	-	-0.282	-	-0.431	-0.404	-0.430	-	-	-	-
31	Mougeotia_16um.	-0.209	-	-	-	-	-	-	-	-	-	-	-	-	-
32	Mougeotia_20um.	-	-	-	-	-	-	-	-0.369	-0.343	-0.278	-	0.216	0.320	-
33	Mougeotia_25um.	-	-	-	-	-	-	-	-0.388	-0.363	-0.363	0.371	0.372	-	-
34	Microthamnion	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	Microspora_8um.	-0.274	-	-	-	-	-0.323	-	-	-	-	-	-	-	-
36	Microspora_11um.	-	-	-	-	-	-	-	-	-	-	-	-0.359	-0.375	-0.741
37	Microspora_14um.	-	0.325	0.296	0.284	-	-	-	-	-	-	-	-	-	-
38	Microspora_22um.	-	-	-	-	-	-	-	-0.228	-	-	0.359	0.335	0.333	-
39	Geminella_8um.	-0.261	-	-0.245	-	-	-	-	-0.319	-0.331	-0.286	-	-	-	-
40	Geminella_11um.	-	-	-	-	-	-	-	-0.278	-0.126	-0.363	-	0.270	-	-
41	Hormidium_5um.	-	-	-	-	-	-	-	-	-	-	-0.402	-0.338	-0.323	-
42	Hormidium_8um.	0.339	-	-	-	-	-	-	-	-	-	-	-	-	-
43	Ulothrix_8um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	Ulothrix_11um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	Ulo./scaly_11um.	0.210	-	-	-	-	-	-	-	-	-	-	-	-	-
46	Ulothrix_14um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	Ulothrix_17um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	Ulothrix_28um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	Ulothrix_36um.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	SIGNIFICANT @ 0.1%	0.193	0.165	0.273	0.165	0.312	0.199	0.312	0.197	0.198	0.205	0.299	0.196	0.196	0.298

Non-significant correlations with either untransformed or Log_{10} data are found for absorbance, TON and SiO_2; the Log_{10} (A_{400}) and (A_{250}) data give significant correlations at 1%.

The relationship between total species number and environmental variables is also shown in Fig 27 a, b, where the first-order (linear) regression line has been drawn on a scatter plot. Similar results were obtained from correlations between individual species' abundances and Log_{10} transformed environmental variables (Table 51). Square-root transformation gives values of r intermediate between the untransformed and Log_{10} values, and this transformation was therefore rejected.

Non-linear methods are available in CANOCO; these are predicated on the existence of a unimodal response of species to environmental variables. It is thus important that such a response can be shown for at least a proportion of species.

The proportion of samples showing a positive abundance score for a given species has been plotted against pH (Fig. 53) as a major environmental variable, and the one for which the most complete data is available. Out of a selected list of common species, a number show a unimodel distribution, while others show a pattern which is equivocal, possibly showing a peak towards the higher pH end of the distribution (pH 7.5-8.0). For the latter species a linear analysis might be appropriate. However for the community as a whole the use of methods based on a unimodal model appears to be justified.

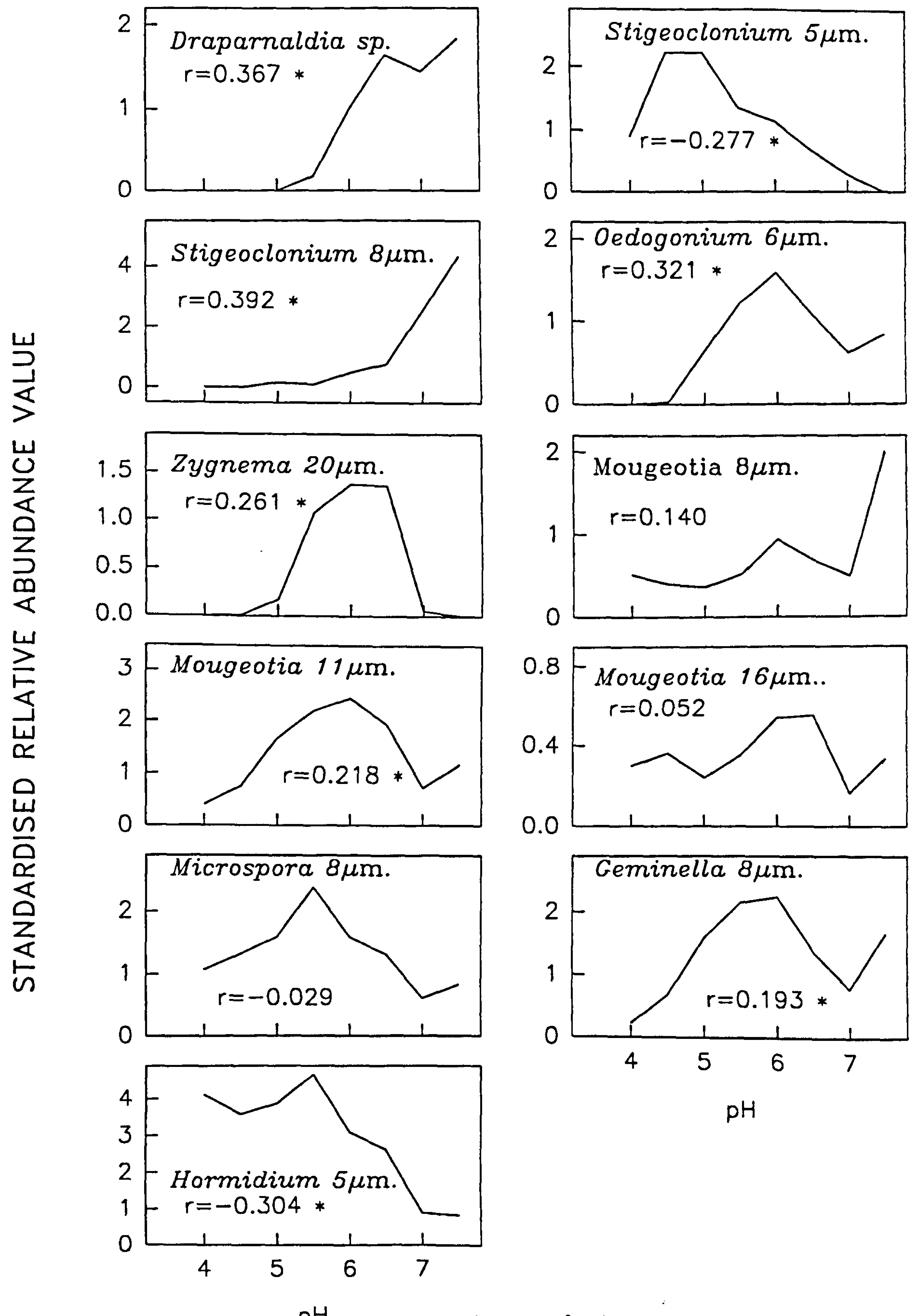
4.7.2 Linear correlations between species abundance and environmental variables

A large Pearson's r value implies a strong linear relationship between two variables, while a low r implies either no relationship at all exists, or that the relationship is non-linear.

Pearson's r has been calculated (MINITAB) between the majority of species data (excluding only some rare taxa) and environmental variables, both untransformed and subjected to Log_{10} transformation. If transformation of the variable leads to consistently higher correlations with the majority of species, that transformation should be applied in subsequent analyses.

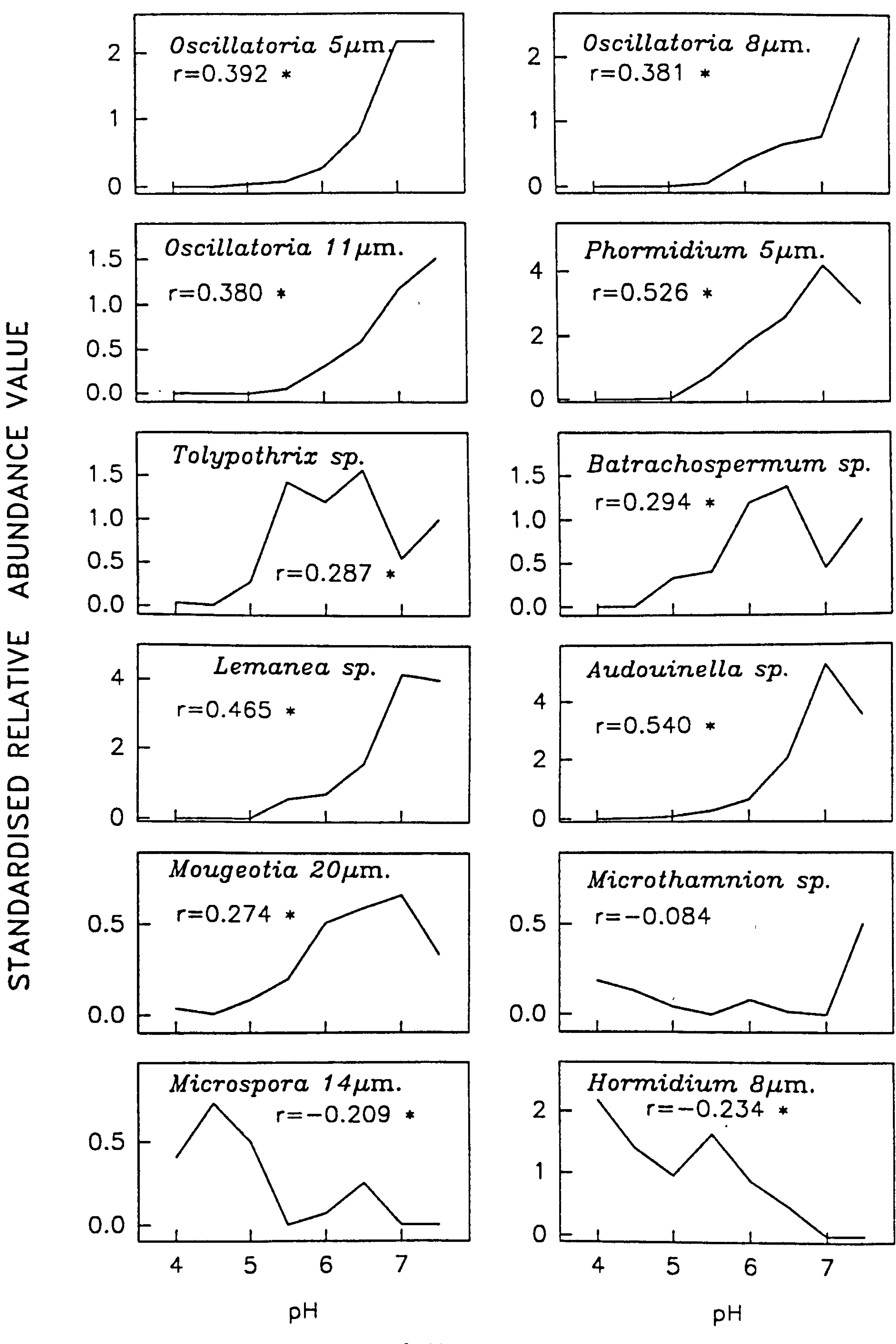
The highest r values are presented in Tables 49 and 51, showing the maximum obtainable correlations between species and variables. Most values are less than 0.5 which ordinarily implies a poor correlation between two variables. However it is to be expected that the distribution of species will be affected by several factors acting at once, possibly with interactive effects, so that even quite low r values may still reveal important relationships, where the correlation is statistically significant. All values of r shown in the table exceed the critical value at the 0.1% level.

Fig.53a:– PLOTS OF RELATIVE ABUNDANCE OF SPECIES AGAINST pH.



* correlations significant at 0.1%

Fig.53b:– PLOTS OF RELATIVE ABUNDANCE OF SPECIES AGAINST pH.



* correlations significant at 0.1%

Fig.54a:- PLOTS OF RELATIVE ABUNDANCE OF SPECIES AGAINST A_{250}.

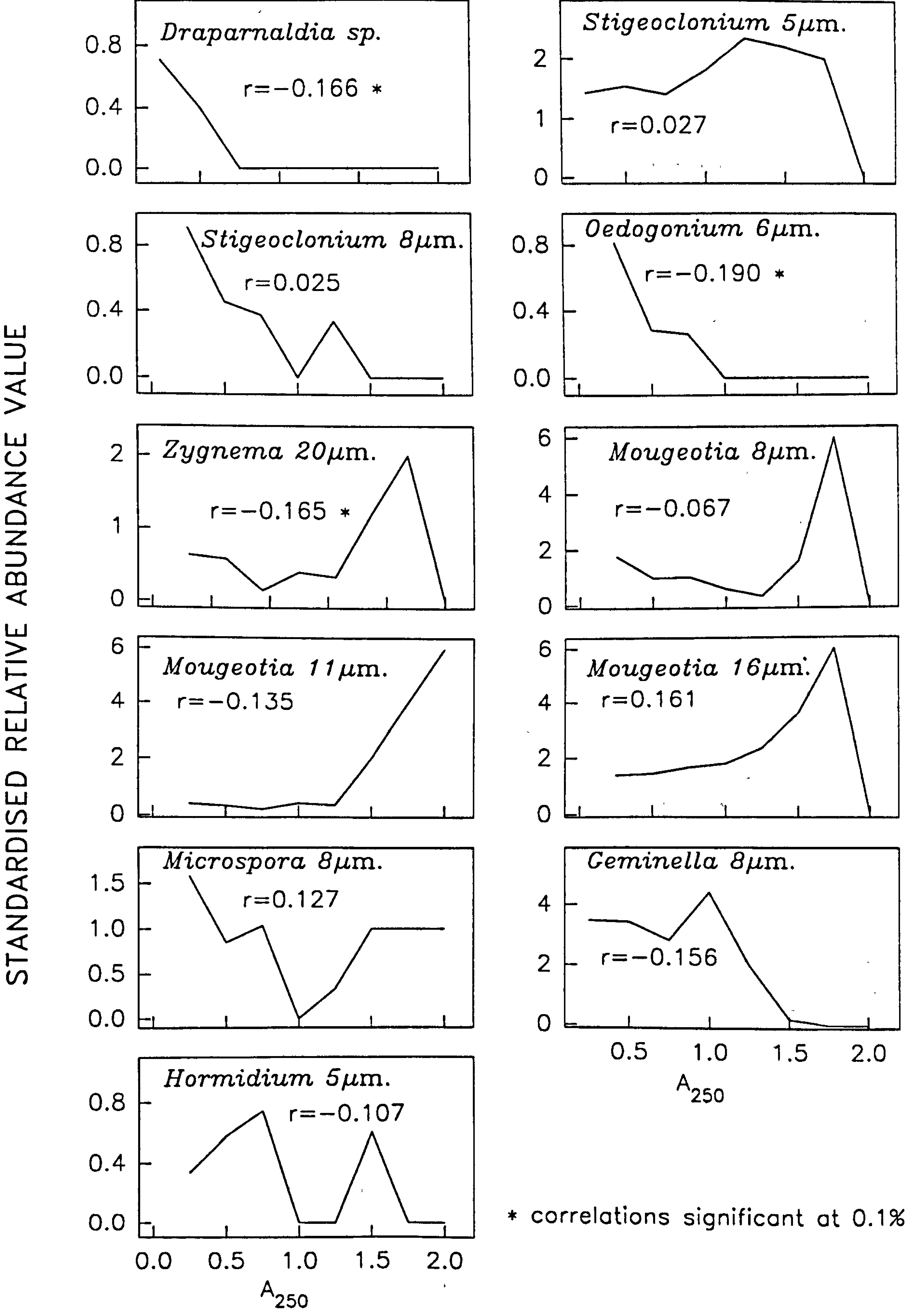
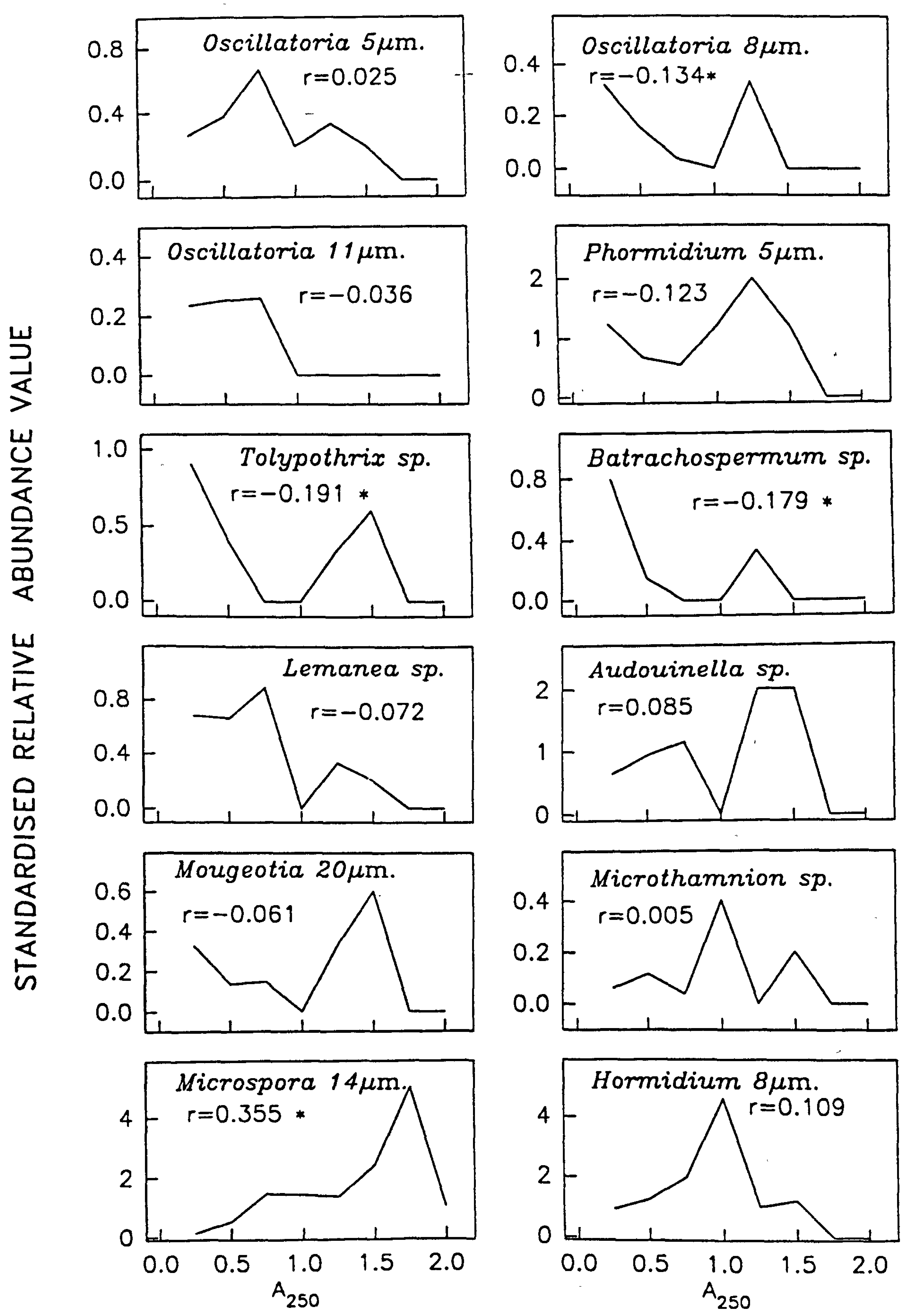


Fig.54b:- PLOTS OF RELATIVE ABUNDANCE OF SPECIES AGAINST A_{250}.



* correlations significant at 0.1%

FIG.55a :— PLOTS OF RELATIVE ABUNDANCE OF SPECIES AGAINST TOTAL MONOMERIC Al.

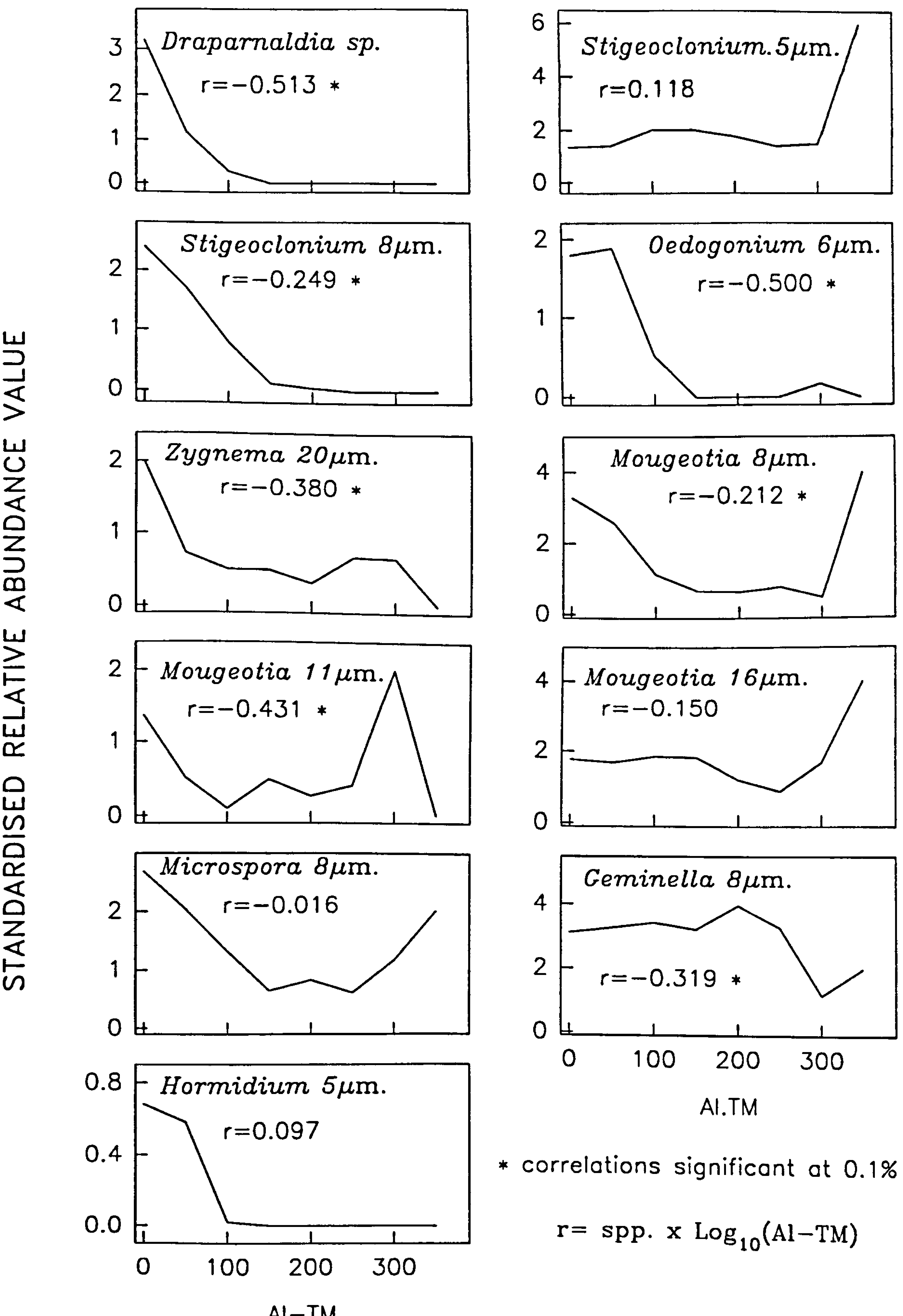
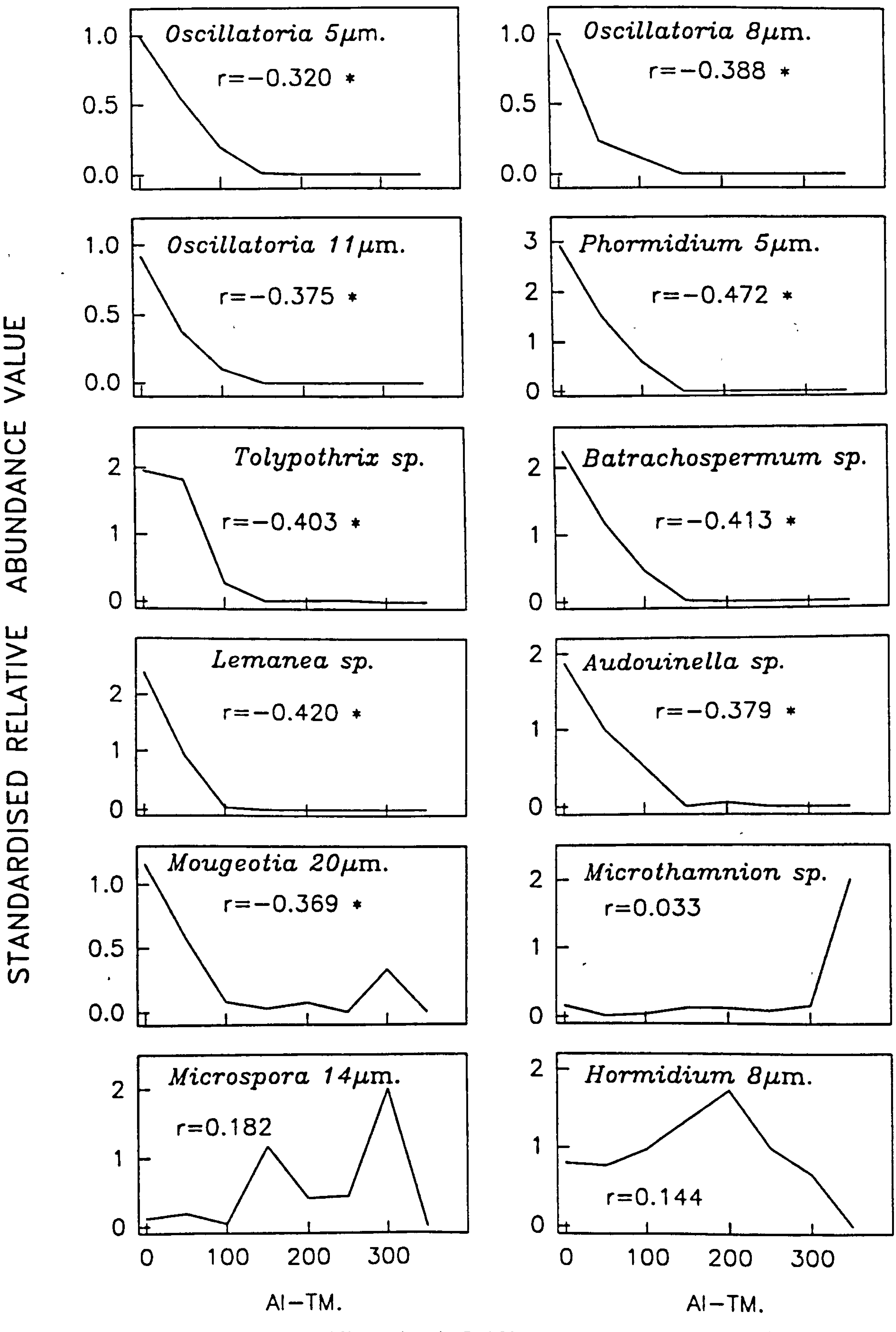


Fig.55b:— PLOTS OF RELATIVE ABUNDANCE OF SPECIES AGAINST TOTAL MONOMERIC Al.



* correlations significant at 0.1% ; r=spp.xLog$_{10}$(Al.-TM)

A linear relationship between a species and an environmental variable means that there should be a steady increase (or decrease) in abundance of that species with an increase of the variable over the range of values of the variable encountered.

The actual distribution patterns of selected species with respect to environmental variables has been investigated by plotting abundance against values of the variable. The values of variables have been grouped in bands and the species abundancies within each band summed, then standardized by dividing by the number of observations of that value of the variable.

Plots have been prepared in this way of species distribution against pH, A_{250} and total monomeric aluminium (Figs. 53-55). No transformation of variables was carried out before constructing these plots, as the sole purpose was to obtain an idea of the nature of the relationship, and transformation would not have altered the basic shape of the curve obtained. Pearsons' r is shown on each plot.

Those species showing a unimodal distribution should not also have a large r with that variable; those with a large r value should show a maximum abundance towards one end of the variable's distribution. By and large the results are in accordance with this expectation, implying that important information about the relationships between species and environment may be obtained by both linear analyses and those based on a unimodal distribution model.

4.7.3 <u>Canonical Correspondence Analysis</u> (Ter Braak, 1988)

4.7.3.1 Use of CANOCO

This is a direct gradient method, as opposed to indirect gradient methods such as PCA in which species data are used to construct ordination axes which may then be tested for correlations with environmental variables. In CCA the environmental variables are involved in the construction of the ordination diagram from the start.

The results of CCA (and Detrended CCA) may be exhibited in a biplot, where the species values, site values and environmental variables are present in the same ordination diagram, enabling inferences to be drawn about the relationships existing between species, sites and environmental factors. The lengths of the environmental factors' arrows indicate the relative importance of the factors in explaining the variance in the species data (Ter Braak and Prentice, 1988).

Arrows which are almost parallel indicate that these environmental factors may be highly correlated and thus each will have no unique contribution to the ordination. All but one could be removed from the analysis without affecting the ordination. The presence of a Variance Inflation Factor (VIF) greater than 20 in the CANOCO output also indicates a high degree of intercorrelation. However there is nothing to indicate which in a set of intercorrelated variables are actually responsible for the community composition.

The ordination diagram is not hampered by high correlations between species or between environmental variables (Ter Braak, 1987).

The final analyses selected have been carried out on the three matrices and both abundance and pooled P/A data sets using as far as possible the same conditions and variables, with the restriction that in the case of Matrix A only 8 variables can be subjected to a canonical analysis, as opposed to 13 for B and C (2 less than the number of sites). The results are shown in Figs. 56-63.

The robustness of the ordinations obtained can be investigated by comparing matrices A, B and C as both abundance and pooled presence-absence data. The abundance data should contain more information but are based on estimated relative abundance; the pooled presence/absence data ought to be free of any bias due to estimation. Both types of data however will span a much narrower range of values than would true abundance data based on counts of cell number.

Bias may be introduced by the presence of rare species (Ter Braak, 1988) at the edge of the species distribution and a correction can be introduced in CANOCO by downweighting rare species. The effect of this downweighting was found to be minimal in presence-absence data Matrix B, but applied to the abundance data it reduced the spread of the species sites and environmental variables along Axis 2. Downweighting of rare species has therefore been applied to all the ordinations shown, in order to make them comparable. The choice of environmental factors to be included in the ordination was made on the basis of the statistics shown in the summary tables (Tables 52-54). Removal of factors was carried out systematically to produce low VIFs as values greater than 20 indicate a high degree of intercorrelation between variables, also evident from the correlation matrix (Ter Braak, 1988).

FIG.56: CANOCO Biplot: DCCA on Matrix A abundance data

SPECIES SCORES

>AUDOU.6
>STIGONE
>MOUG.20
>STIGEO8
>ULO/S11
>MICRO11
>GENIN.8 >OEDOG.6 >MOUG.25 OSC.8/0.11u
>HORM.5u OEDOG30 < ^>OSC.1/0.14/PHORM.8/SPIRO.20/S.32/ULOTH.14u
>MOUG.11 PHORM.3 BATR >OSC.5/AUDOUIN.
HORM.8u-<>MICROS8 >GENIN11 >OEDOG14
MOUG.5u/ULOTH.8< PHORM.5 <>DRAPARN
ULOTH11 <v >MICROTH
MICRO14 <STIG.5 >MOUG.16
>MICRO22
ZYGN.20 <>OSC.2u/TOLYPOTH/TOLY.EPIPH/ULOTH.17u
pH

SAMPLE SCORES

a = 9/10-1/10-2/11-2
> 2
14-2 < > 5
>a v > C-2
> 11-1 6

BIPLOT SCORES OF ENVIRONMENTAL VARIABLES

AREA
'FLOW'
pH INS
TON

FIG.57: CANOCO Biplot: DCCA on Matrix A pooled p/a data

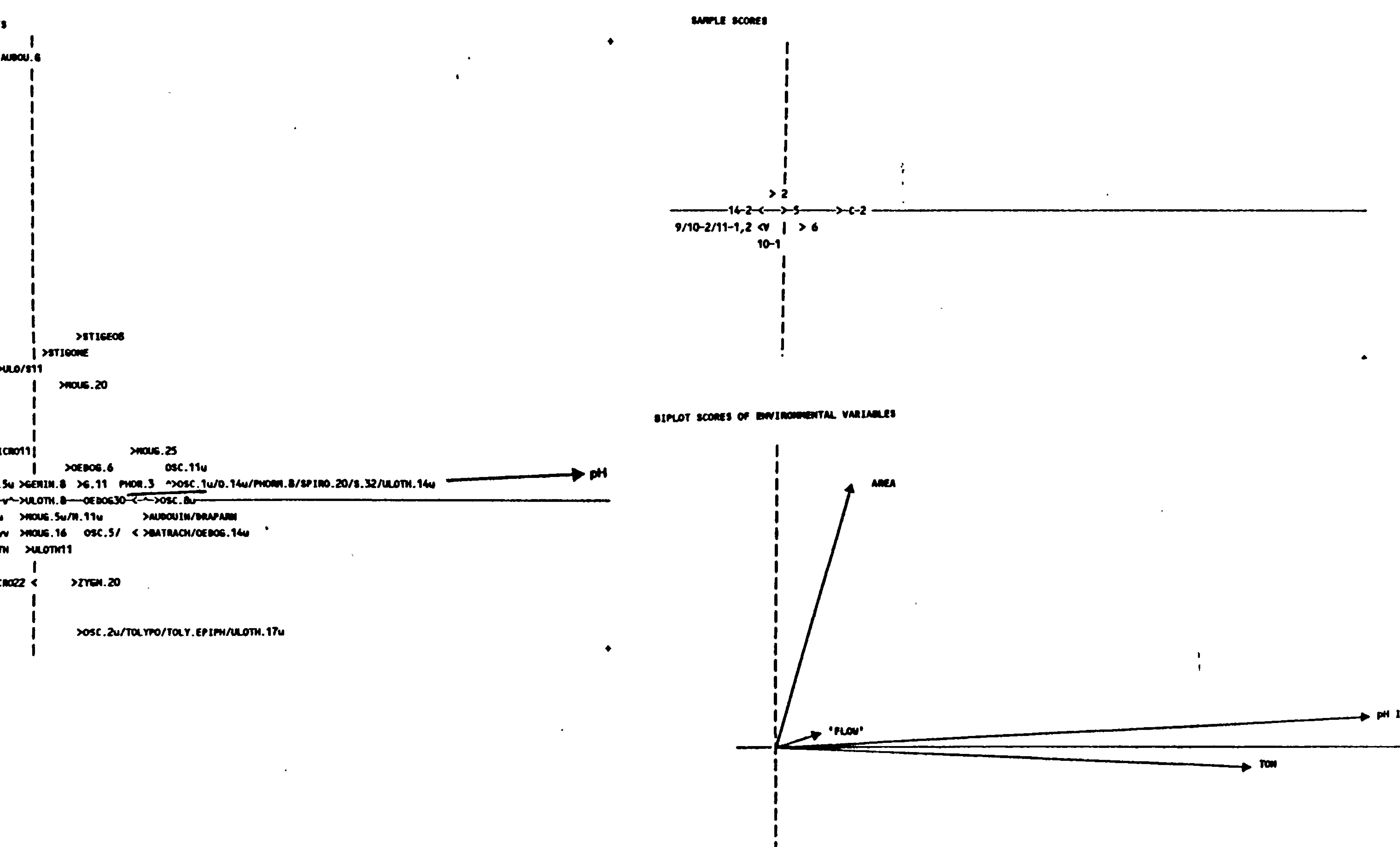


FIG.58: CANOCO Biplot: CCA on Matrix B abundance data

FIG.59: CANOCO Biplot: CCA on Matrix B pooled p/a data

FIG.60: CANOCO Biplot: DCCA on Matrix B abundance data

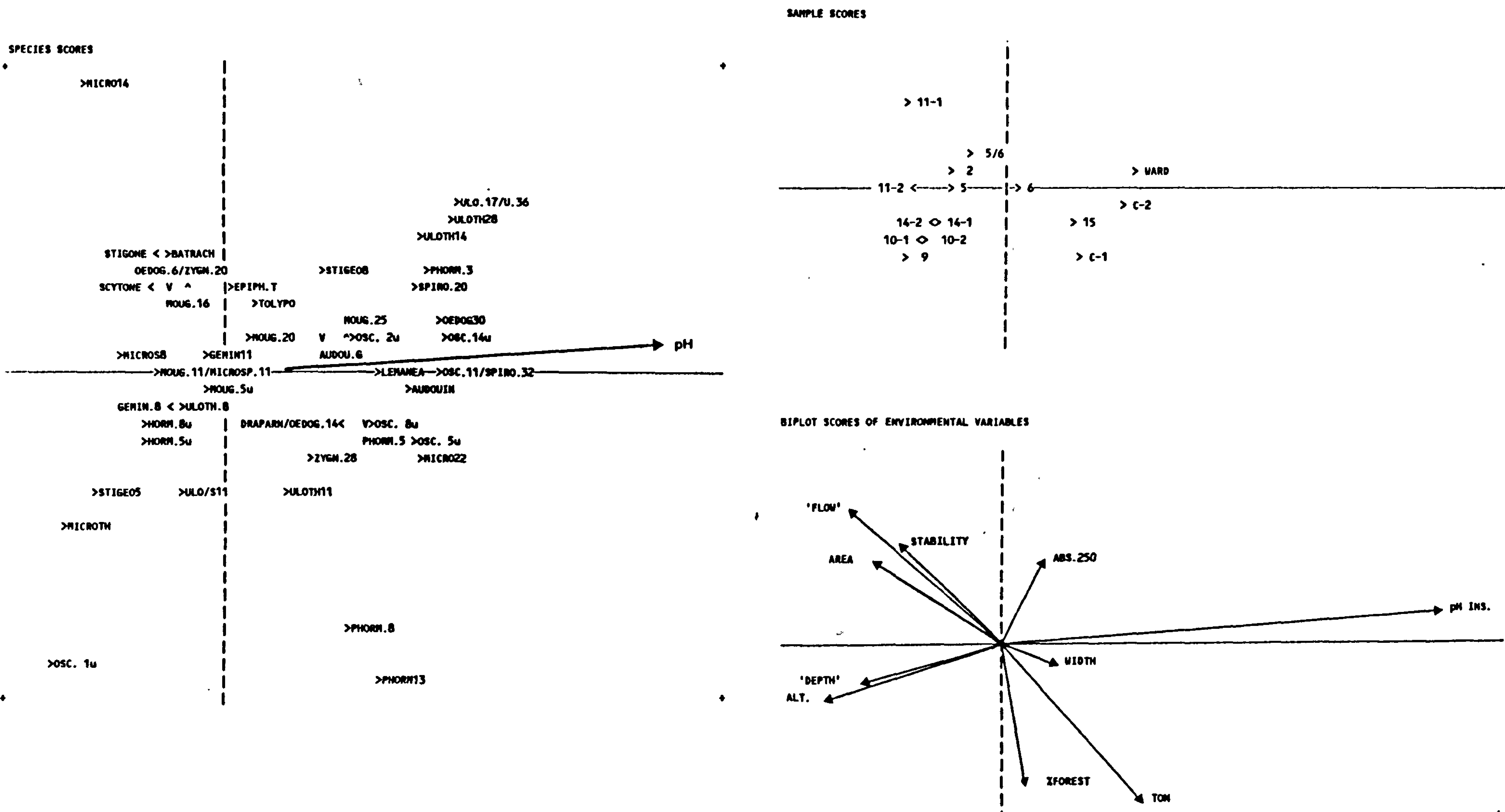


FIG.61: CANOCO Biplot: DCCA on Matrix B pooled p/a data

SPECIES SCORES

>PHORM.8
>PHORM13
>OSC. 1u
>MICROTH
>MICRO22
DRAPARNAL AUDOUIN
>STIGEO.5 >ULO/S11 >ULOTH11 ^ ^ v >OSC.11u
>HORM.5u PHORM.5< OSC.8u
ULOTH.8 >OSC. 2u
HORM.8u < ^ >MOUG.5u >OSC. 5u
GENIN.8-<->MOUG.11-MOUG.25-<>OEDOG14 >SPIRO32
pH
>GENIN11 >ZYGN.28
MICROS8 < >MICRO11 AUDOU.6 < >LEMANEA
EPIPH.T < >TOLYPO PHORM.3 <
MOUG.16 < >OEDOG.6 STIG.8 < OEDOG.30> >OSC.14u
BATRACH < >ZYGN.20 >MOUG.20 >SPIRO20
>MICRO14
>SCYTONE
>STIGONE
>ULOTH14
>ULOTH28
>ULOTH.17/U.36

SAMPLE SCORES

> C-1
10-1/10-2
9 <^ 14-1 6
14-2-<^-v- ~ V-> C-2
11-2 <v 2< 5 15
11-1 > 5/6 > WARD

BIPLOT SCORES OF ENVIRONMENTAL VARIABLES

%FOREST
TON
ALTITUDE
WIDTH
'DEPTH'
pH INS.
STABILITY
'FLOW'
AREA
ABS.250

FIG.62: CANOCO Biplot: DCCA on Matrix C abundance data

SPECIES SCORES

>STIGONE
>EPIPH.T
ZYGN.20 <
>BATRACH
>TOLYPO
>STIGEO8
ULOTH17 <>ULOTH36
>ULOTH28
>ULOTH14
>PHORM.3
>SPIRO20
>OEDOG30
>OEDOG.6
>SCYTONE
>AUDOU.6
>OSC. 2u
>MOUG.11
>GEMIN11
pH
MICRO58 <
>MICRO11
>MOUG.20
>MOUG.16
LEMANEA-<
>AUDOUIN
HORM.5u
STIGEO5 <
^ >GEMIN.8
>MOUG.25
>MOUG.5u
>OSC.14u/MICRO.22u
>MICRO14
>HORM.8u
>ULOTH11
ULOTH.8 <>ULO/S11
>OSC. 5u
DRAPARN <
V V
>OSC.11u/SPIRO.32
PHORM.5u
OSC.8u
>OEDOG14
>MICROTH
>ZYGN.28
>OSC. 1u
>PHORM.8
>PHORM.13

SAMPLE SCORES

> 5/6
> WARD
> 6
2 <> 5
11-2-<---> 14-1
11-1 <VV> 14-2
9 10-1/10-2
> C-2
> 15
> C-1

BIPLOT SCORES OF ENVIRONMENTAL VARIABLES

'FLOW'
ORDER
AREA
ABS.250
pH INST.
STABILITY
ALTITUDE
WIDTH
'DEPTH'
%FOREST
TON

FIG.63: CANOCO Biplot: DCCA on Matrix C pooled p/a data

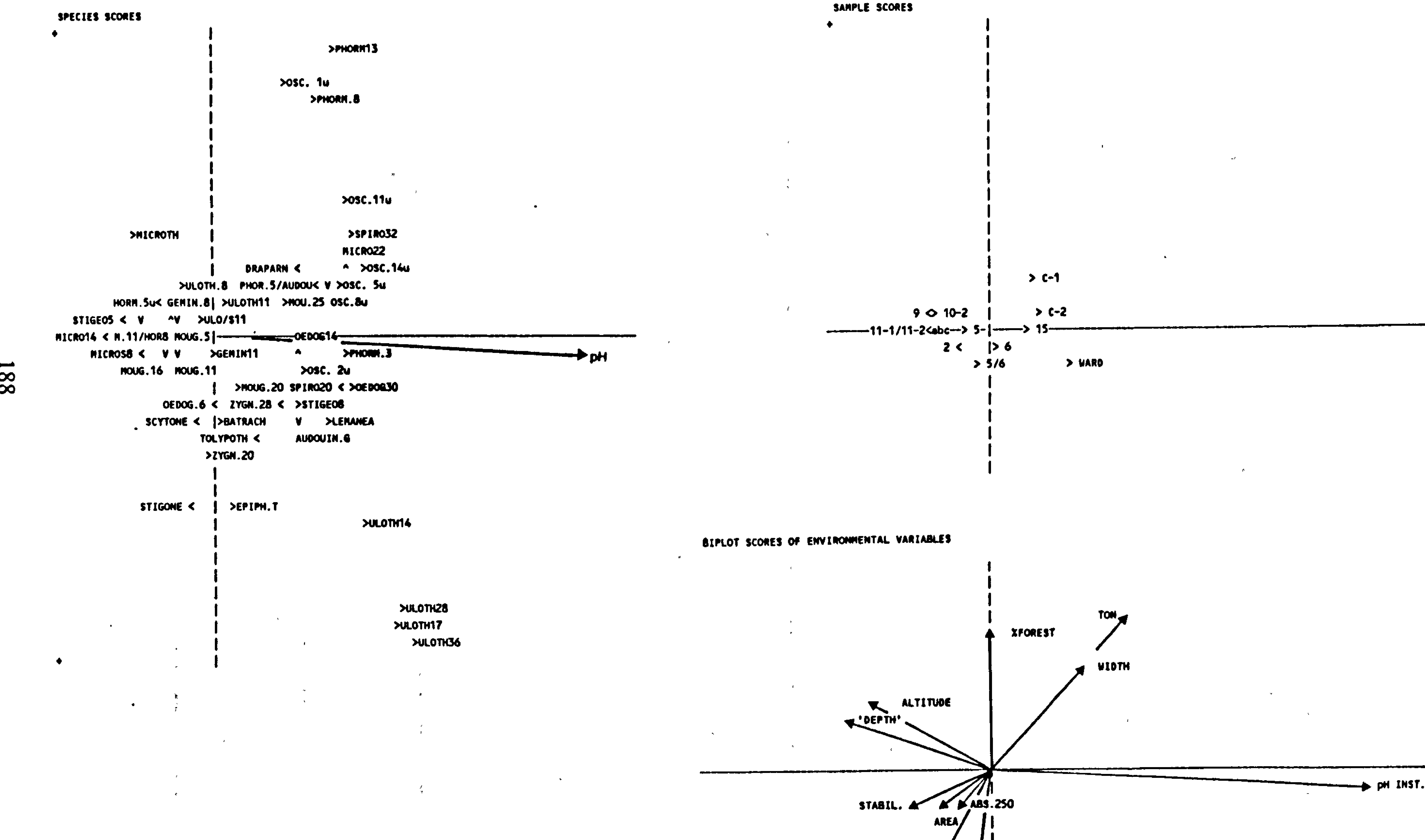


TABLE 52
SUMMARY STATISTICS:
CANONICAL CORRESPONDENCE ANALYSES

MATRIX: DATA TYPE:	A Abundance	A P/A	B Abundance	B P/A	C Abundance	C P/A
VARIABLE	(a) VARIANCE INFLATION FACTOR					
pH	3.44*	3.97*	6.71*	5.69	4.54**	4.41*
'Flow'	-	-	5.99	7.04	5.15*	4.67*
'Depth'	3.04	3.73	3.92	5.46	3.45	3.29
A_{250}	-	-	5.27	5.78	3.85	4.10*
TON	5.66	7.23	6.81	12.67*	8.34	10.87
Altitude	-	-	6.99	3.26	2.78	2.52
Area	3.98	4.57	7.62	6.90	6.24	8.36
% Forest	9.36	9.68	7.56	10.82*	11.80	16.16
Order	6.07*	7.01*	9.76	-	1.82*	1.44*
Width	-	-	6.83	8.66	6.44	6.20
Stability	-	-	3.80	5.02*	4.45	3.64
	(b) EIGENVALUES					
Axis 1	0.446	0.361	0.543	0.414	0.518	0.391
2	0.216	0.167	0.287	0.192	0.264	0.172
3	0.076	0.055	0.184	0.102	0.144	0.084
4	0.055	0.041	0.160	0.086	0.120	0.078
Sum (= trace)	0.848	0.665	1.586	0.972	1.384	0.900
	(c) % VARIANCE ACCOUNTED FOR BY FIRST TWO AXES					
Axis 1	52.6	54.3	34.3	42.6	37.4	45.3
2 + 1	78.1	79.4	52.4	62.4	56.5	62.6
	(d) CORRELATION BETWEEN pH and CANONICAL AXIS 1					
r	0.952	0.963	0.955	0.973	0.952	0.973
	(e) MONTE CARLO PERMUTATION TEST: AXIS 1					
p	0.16	0.09	0.01	0.01	0.03	0.02

* = t value > 2.1 on Axes 1 or 2

TABLE 53
SUMMARY STATISTICS:
DETRENDED CANONICAL CORRESPONDENCE ANALYSES

MATRIX: DATA TYPE:	A Abundance	A P/A	B Abundance	B P/A	C Abundance	C P/A
VARIABLE	(a) VARIANCE INFLATION FACTOR					
pH	6.4*	7.5*	5.2**	5.7*	4.5**	4.4*
'Flow'	4.9	5.5	6.9*	7.0	5.2*	4.7
'Depth'	-	-	5.3*	5.5	3.4	3.3
A250	-	-	5.6**	5.8	3.9**	4.1*
TON	6.7	8.4	11.8**	12.7	8.3*	10.9*
Altitude	-	-	3.2	3.3*	2.8	2.5*
Area	1.27*	1.23*	6.9*	6.9	6.2	8.4
% Forest	-	-	10.8	10.8*	11.8	16.2*
Order	-	-	-	-	1.8**	1.4*
Width	-	-	7.8	8.7	6.4*	6.2
Stability	-	-	5.9**	5.0*	4.4	3.6
	(b) EIGENVALUES					
Axis 1	0.448	0.362	0.545	0.413	0.518	0.391
2	0.067	0.054	0.179	0.110	0.175	0.111
3	0.081	0.038	0.161	0.094	0.119	0.075
4	-	-	0.025	0.017	0.027	0.017
Sum (= trace)	0.71	0.58	1.51	0.97	1.38	0.90
	(c) % VARIANCE ACCOUNTED FOR BY FIRST TWO AXES					
Axis 1	59.3	62.1	36.0	42.6	37.4	43.5
2 + 1	71.6	71.3	47.8	53.9	50.0	55.8
	(d) CORRELATION BETWEEN pH and CANONICAL AXIS 1					
r	0.95	0.71	0.96	0.97	0.95	0.97
	(e) MONTE CARLO PERMUTATION TEST: AXIS 1					
p	0.02	0.02	0.01	0.01	0.03	0.02

* = t value > 2.1 on Axes 1 or 2

TABLE 54
SUMMARY STATISTICS:
DETRENDED CANONICAL CORRESPONDENCE ANALYSES
OMITTING SPECIES 1 (*Oscillatoria* 1μm.)

MATRIX: DATA TYPE:	A Abundance	B Abundance	C Abundance
VARIABLE	(a) VARIANCE INFLATION FACTOR		
pH	3.44	5.22	4.54
'Flow'	-	6.93	5.15
'Depth'	3.04	5.30	3.44
A_{250}	-	5.56	3.86
TON	5.63	11.77	8.33
Altitude	-	3.15	2.78
Area	3.98	6.87	6.24
% Forest	9.36	10.77	11.80
Order	6.07	-	1.82
Width	-	7.81	6.43
Stability	-	5.95	4.45
	(b) EIGENVALUES		
Axis 1	0.443	0.545	0.518
2	0.087	0.179	0.174
3	0.015	0.161	0.119
4	0.066	0.025	0.029
Sum (= trace)	0.844	1.513	1.383
	(c) % VARIANCE ACCOUNTED FOR BY FIRST TWO AXES		
Axis 1	52.6	36.0	37.4
2 + 1	62.8	47.8	50.1
	(d) CORRELATION BETWEEN pH and CANONICAL AXIS 1		
r	0.952	0.958	0.952
	(e) MONTE CARLO PERMUTATION TEST: AXIS 1		
p	0.17	0.01	0.03

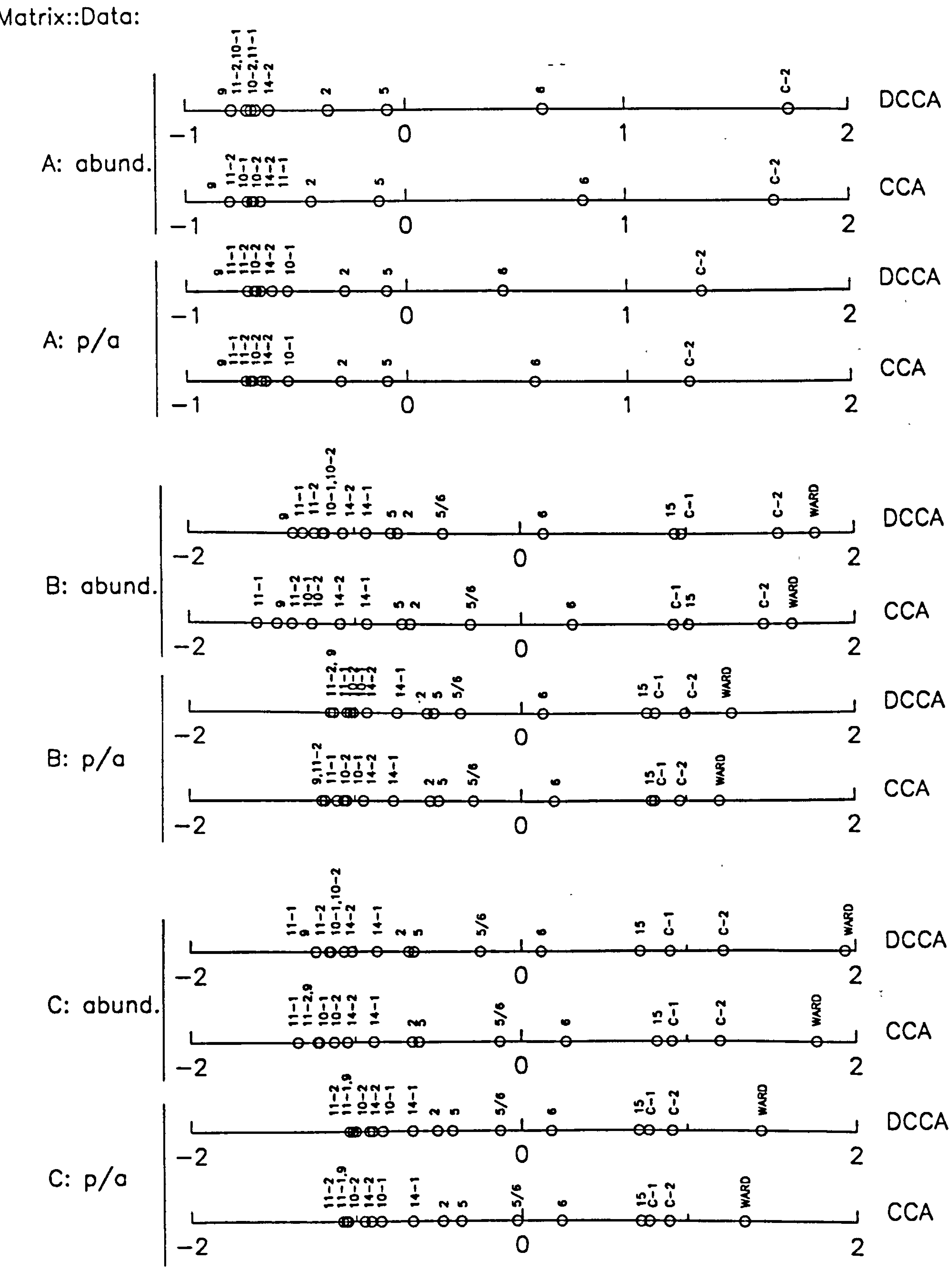
* = t value > 2.1 on Axes 1 or 2

Analyses were rejected if no t values (of the canonical correlations) greater than 2.1 in absolute value were obtained (Ter Braak, 1988). However in no analysis did all the variables have t values > 2.1. In the table, two asterisks indicate a t > 2.1 on both axis 1 and 2. The importance of the canonical axes is indicated by the eigenvalues. In all cases Axis 1 has an eigenvalue considerably higher than the other axes. The first two axes account for 50% or more of the variance. However this is to some extent a function of the number of variables included in the analysis, hence the higher percentages found with Matrix A. The eigenvalues are a better criterion for judging the importance of the axes (Ter Braak, 1988).

A Monte Carlo permutation test was carried out in CANOCO on the ordinations obtained and any analysis repeated (using different combinations of environmental variables) if the significance of the first canonical axis was not greater than 5% ($p < .05$). In Matrix A high values of p are obtained with even a reduced set of variables (Tables 51 and 53), but if the list is reduced to just the four shown in Table 52, a p of 0.02 is obtained with both abundance and p/a data, analysed by CCA or DCCA. Thus a significant analysis can be obtained at the cost of losing information on some of the variables. The results of six sets of analyses using DCCA(Table 53) and two using CCA on matrix B (Table 52), are presented in the form of ordination diagrams in which the species and site values and environmental variables' arrows are plotted on canonical axes 1 and 2 (Figs. 56-63).

In a biplot, the environmental arrows are superimposed on the ordination diagrams of species and site values. For clarity in Figs. 57-64, the information has been presented separately, but with the pH arrow superimposed on the species' values. In each figure, the pH arrow is very close to canonical axis 1, indicating that the distribution of species and sites along axis 1 is mainly dependent on acidification related variables. In some figures the diagrams are mirror images of others, since the axes are in fact artificial constructs. This does not matter but it makes comparison of the figures more difficult. The recalculated species and site values on the pH arrow (Figs. 64-66) make comparisons easier.

FIG.64: SITE VALUES ON pH VECTOR, DERIVED FROM (DETRENDED) CANONICAL CORRESPONDENCE ANALYSES.



SITE VALUES ON pH ARROW FROM ORDINATION

[Obtained by dropping a perpendicular from each site point to the pH arrow]

FIG.65: SPECIES VALUES ON pH VECTOR, DERIVED FROM CANONICAL CORRESPONDENCE ANALYSES.

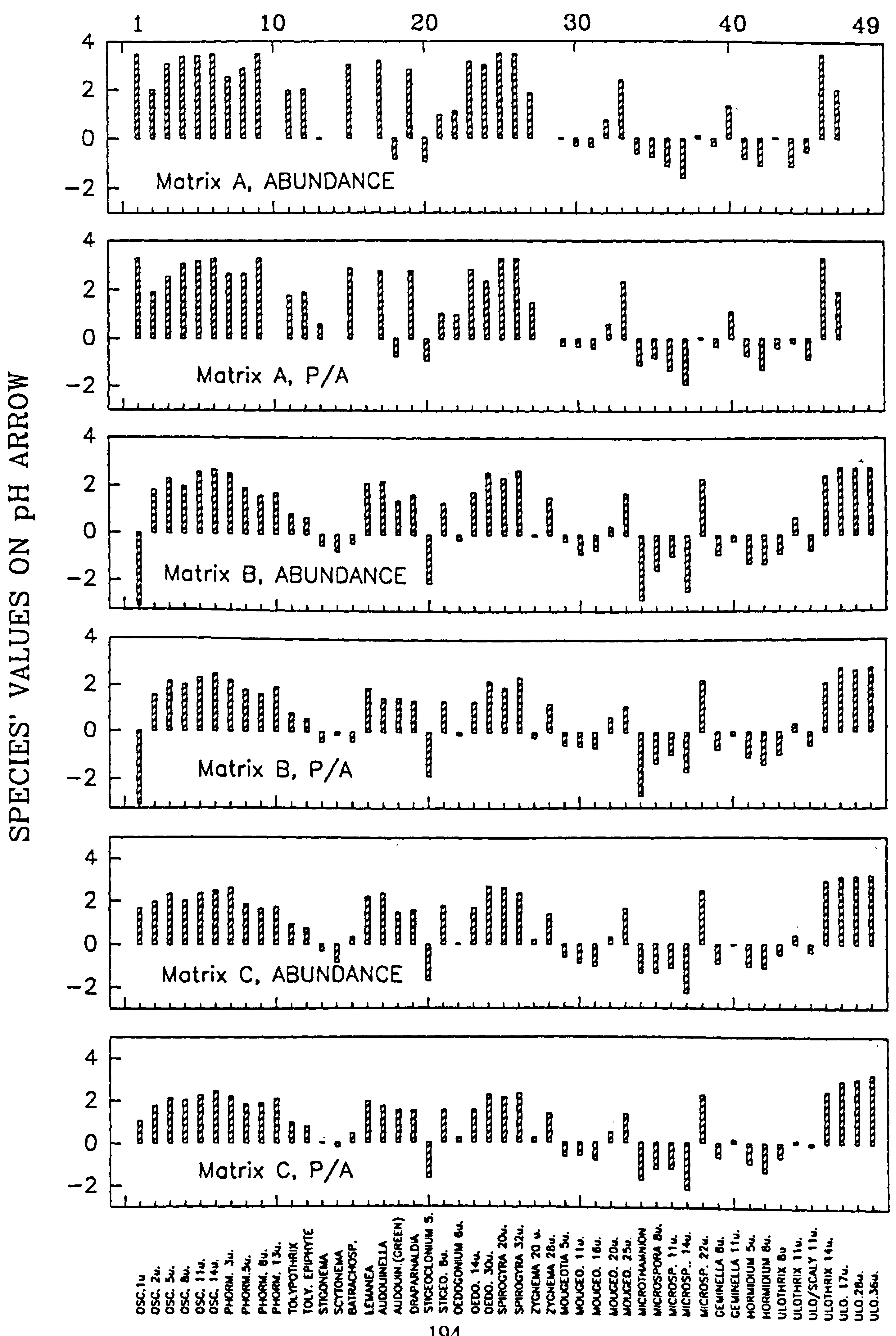
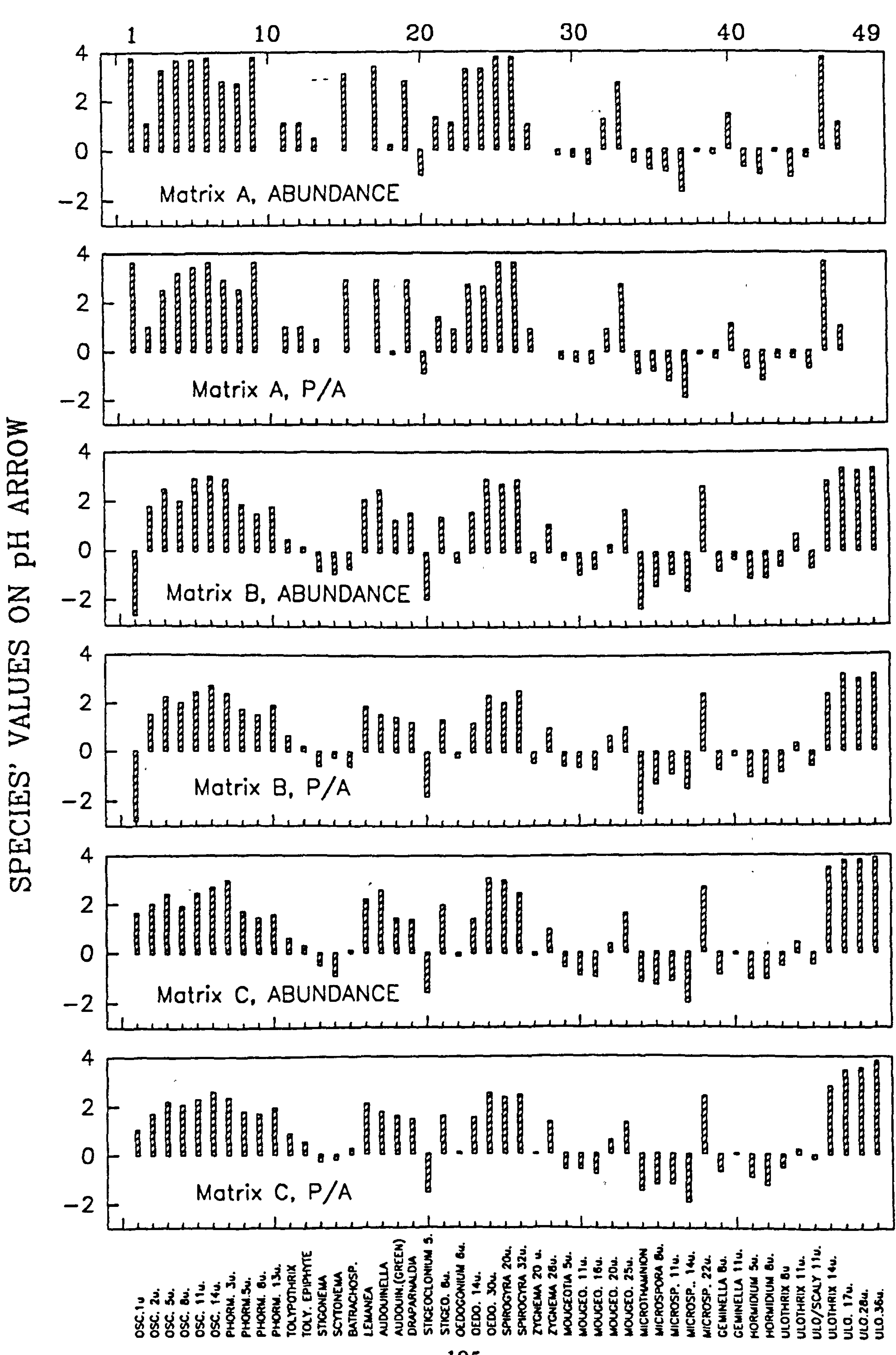


FIG.66: SPECIES VALUES ON pH VECTOR, DERIVED FROM DETRENDED CANONICAL CORRESPONDENCE ANALYSES.



4.7.3.2 Variables excluded from analysis by CANOCO

Some variables were excluded from the analysis because of insufficient data or other reasons for considering them unreliable as discussed below.

(a) 'Flow' is the depth gauge reading on site. It takes no account of the other hydrological factors which determine the current velocity and thus is not strictly comparable between sites. Furthermore alterations in the stream beds have occurred since the installation of the gauges so that the zero mark lies beneath the stream bed in some cases. The 'Flow' data was therefore adjusted by subtracting from each the minimum reading encountered, giving the variable 'Depth', which is thus the mean fluctuation in water depth.

(b) Temperature was measured on site during sampling. The measured temperature is influenced by time of day, being related to insolation. The same order of site visitation was used on most occasions, so that the time of sampling each site can be predicted within an hour or two. It is found that when the mean temperature is plotted against G.M.T., a reasonable straight-line regression is obtained (Fig. 25b), implying that the spot-measured temperature is not a reliable measure of real differences between sites. Measurements of maximum and minimum temperature were too infrequent to be of value.

(c) PAR was measured only at certain sites as insufficient instruments were available and some sites were considered too public to leave an unattended instrument for several weeks. The instruments were furthermore not available during the earlier part of the investigation. Therefore although calculated values of PAR have been used in Pearson correlation analysis there were insufficient sites with measured 'percent of open' values to enable PAR to be usefully included in CANOCO.

(d) Measurements of silica were carried out relatively infrequently and therefore mean values are not available for some sites.

(e) Phosphate is to a lesser extent subject to the same problem, as the distribution of values for PO_4 is biased because analyses were carried out by SOAFD more frequently following fertilization of the catchment by the Forestry Commission.

(f) Variables such as alkalinity, Ca^{2+}, Mg^{2+}, Al and absorbance had to be dropped from many of the analyses because of their contribution to high VIFs. These are clearly *a priori* and as implied in these analyses, acidification-related variables. However one variable is able to represent the whole group in terms of the effects of acidification on species distribution. This role has been allotted to pH, but several other variables might equally well have been chosen. For practical purposes however pH is a good choice; it is more easily measured than the others and hence more data are available, and it is a direct measure of the changes implicit in the term 'acidification'.

4.7.3.3 Transformation of variables

Log_{10} transformation is indicated for forms of Al (see section 4.6.1), but these have been excluded on the basis of high intercorrelation with pH, so no transformations have been applied to data for analysis by CANOCO.

4.7.3.4 Results of analyses

In all analyses pH is highly correlated with Canonical axis 1 (Tables 52-54) and in consequence the arrow lies very close to axis 1 in the biplot. The pH arrow is the longest one in the biplot and thus the most important variable in explaining the distribution of species (Ter Braak and Prentice, 1988).

The second important fact to emerge from an inspection of the biplots is that the distribution of the sites along the pH arrow is in all cases virtually identical to the order of sites in Table 46, where they are ranked by mean pH, and to the groupings carried out by CLUSTER.BAS (abundance data) and NASSOC.BAS (presence-absence data). This is shown more clearly in Fig. 64 where the site values on the pH vector have been recalculated and plotted along a single axis, for all matrix and data combinations. The distribution, clearly separating the acidic burns 9, 10, 11 and 14 from the circumneutral Ward, Corrie, 6 and 15, is in fact remarkably stable and was obtained also in analyses where other variables were included, contributing to VIFs exceeding 100. These variables contribute little additional information to the ordination but they do not upset the canonical coordinates (Ter Braak, 1988). Differences in the distribution of species on the diagrams have however been detected and it is for this reason that a common set of variables has been employed as far as possible in comparing the six matrix/data combinations (Tables 52, 53).

The results of these analyses may be most readily compared by consulting Figs. 65 and 66, where the recalculated species values on the pH arrow have been plotted for all matrix/data analysis combinations. In these figures the recalculated species values are presented as a bar chart with the species in the same order to facilitate comparison of the patterns. The value (height of the bar) represents the position on the pH arrow occupied by each species. This is the weighted average of the species occurrence with respect to pH. The value is thus a function of the mean pH at which a given species is found - the mode of the Gaussian distribution curve.

The species values may thus be used as indicator values for inferring environmental pH. Species which occur towards the extremities of the ordination carry more predictive weight, so that in the case of pH, it is the highest and lowest species values which are of the greatest importance. The downweighting of rare species is advisable to prevent rare species, which by chance may fall towards the outside of the ordination, from biasing the result.

The species' values shown in Figs. 65 and 66 are very similar in different matrices and data forms, with the notable exception of '*Oscillatoria* 1 µm' (species 1). This may be due to a tendency to overlook its occurrence in films of organic material prevalent in some of the more acidic streams during the earlier part of the investigation. The finding of an (apparent) cyanophyte in these waters was unexpected (Brock, 1973). Another problem is that these dimensions are near the limits of resolution in field samples which were normally examined at x500 magnification, and this size class may therefore encompass several taxa.

The analyses were re-run with the option allowed in CANOCO for giving zero weight to this species, but apart from the effects on species 1, there was very little difference in the values of the summary statistics (Table 54) and in the configuration of the ordination diagrams (not shown).

Acidification has been noted to result in changes in algal communities in lakes and streams. Both in planktonic and benthic communities, reduction in pH is found to result in a decrease in abundance of diatoms and Rhodophytes and a progressive replacement by blue-greens and then filamentous green algae (Muller, 1980; Morling *et al*, 1985; Stokes, 1986; Marker and Willoughby, 1988; Findlay and Kasian, 1990; Havens and Heath, 1990). These changes are found whether acidification occurs 'naturally', i.e. as a slow process due to catchment acidification, or artificially in the course of experimental manipulation. While some workers find an increase in biomass on acidification (Hendrey, 1976; Muller, 1980; Mulholland *et al* , 1986), others find no change (Arnold *et al* , 1981; Howell *et al* , 1990; Molot *et al* , 1990) or even a decrease (Nalewajko and O'Mahony, 1989).

Schindler (1987) concluded that stress, including acidification, does not lead to changes in production, nutrient cycling or decomposition, but does cause changes in species composition which are thus better indicators of disturbance. Marker and Willoughby (1988) concluded that the increased biomass reported in acidified streams is more apparent than real, due to the different types of algae present.

TABLE 55

RANKING OF SPECIES ALONG AXIS 1 (High -> Low pH)

1. *Ulothrix* 36 µm.
2. *Ulothrix* 28 µm.
3. *Ulothrix* 17 µm.
4. *Ulothrix* 14 µm.
5. *Oscillatoria* 14 µm.
6. *Spirogyra* 32 µm.
7. *Oedogonium* 30 µm.
8. *Microspora* 22 µm.
9. *Oscillatoria* 11 µm.
10. *Spirogyra* 20 µm.
11. *Phormidium* 3 µm.
12. *Oscillatoria* 5 µm
13. *Phormidium* 13 µm.
14. *Oscillatoria* 8 µm.
15. *Lemanea*
16. *Phormidium* 8 µm.
17. *Phormidium* 5 µm.
18. *Audouinella*
19. *Oscillatoria* 2 µm.
20. *Draparnaldia*
21. *Stigeoclonium* 8 µm.
22. *Audouinella* (green)
23. *Oedogonium* 14 µm.
24. *Mougeotia* 25 µm.
25. *Oscillatoria* 1 µm.
26. *Zygnema* 28 µm.
27. *Tolypothrix*
28. Epiphyte of *Tolypothrix*
29. *Mougeotia* 20 µm.
30. *Batrachospermum*
31. *Ulothrix* 11 µm.
32. *Oedogonium* 6 µm.
33. *Geminella* 11 µm.
34. *Zygnema* 20 µm.
35. Scaly *Ulothrix* 11µm.
36. *Stigonema*
37. *Scytonema*
38. *Ulothrix* 8 µm.
39. *Mougeotia* 8 µm.
40. *Mougeotia* 11 µm.
41. *Geminella* 8 µm.
42. *Mougeotia* 16 µm.
43. *Hormidium* 5.5 µm.
44. *Microspora* 8 µm.
45. *Microspora* 11 µm.
46. *Hormidium* 8 µm.
47. *Microthamnion*
48. *Stigeoclonium* 5 µm.
49. *Microspora* 14 µm.

There is a greater degree of agreement about the algae most favoured by acidification. Filamentous greens come progressively to dominate below pH 6.0 (Hargreaves *et al*, 1975; Muller, 1980; Turner *et al*, 1987; Marker and Willoughby, 1988; Howell *et al*, 1990; Jackson *et al*, 1990). The genus *Mougeotia* is particularly prevalent as periphyton in the littoral zone of lakes, or forming free-floating masses of metaphyton, leading to the speculation that either light penetration or DIC concentration limits its development in deeper water (Turner *et al*, 1987). *Zygnema* (or *Zygogonium*) is also reported to occur under these circumstances (Jackson *et al*, 1990). In streams the availability of light or DIC may impose less of a limitation on algal growth.

Marker and Willoughby (1988) found in natural streams of differing pH that at circumneutral pH the filamentous genera *Ulothrix*, *Rhodochorton* (= *Audouinella*), *Batrachospermum* and *Lemanea* were common, but at lower pH the Rhodophytes were replaced by blue-greens and *Hormidium subtile*. In contrast, Mulholland *et al* (1986) found that *Rhodochorton* was present at a site with median pH 4.5. In highly acidified water (e.g. affected by mine drainage), filamentous greens including *Hormidium* sp. and some resistant diatoms may be found, down to pH values below 3.0 (Hargreaves *et al*, 1975).

Most of these results accord with the distribution of species with pH found in the L. Ard streams (e.g. Fig. 63), except that *Audouinella/Rhodochorton* is found predominantly in the higher-pH sites (Fig. 53b) and in association with other 'circumneutral' species (Figs. 29-40). However it has been recorded in every stream apart from Burn 11 on at least one occasion (Tables 4-9).

The ranking of species with respect to pH can be derived from CCA analysis. In an analysis where the pH arrow is very close to axis 1, e.g. Fig. 63, the order of species along axis 1 given in the CANOCO output will be effectively the ranking of species with respect to pH (Table 55). This ranking does not lend support to the observation of Havens and Heath (1990) that acidification leads to a shift towards larger-diameter species. Rather the opposite trend appears in the L. Ard streams. This difference may be accounted for by changes in grazing pressure during the 23-day experimental acidification and aluminium spiking experiment of Havens and Heath (1990), in in-lake polyethylene mesocosms.

Sensitive zooplankton may be very rapidly eliminated by such manipulations leading to a community dominated by forms too small to exploit the larger algae, leaving them at a competitive advantage. These zooplankton species do not exist in lotic environments and in any case a longer-term acidification will permit the establishment of a stable algal community which may not be the same as a replacement community following ecosystem disturbance.

If the change in algal species which occurs on acidification is due to a rapid development of species tolerant of the new conditions, i.e. able to exploit a resource left vacant by intolerant species, it is more likely to result in a community dominated by small cell forms, as the maximum specific growth rate is inversely related to cell diameter (Raven and Geider, 1988). Therefore if a change in species composition is due to a shift in grazing pressure, a population of larger cells is likely to result in the short term, but if grazing is not important prior to acidification, the post-acidification population should consist of smaller algal cells. The final, steady-state condition however will depend on the pH-sensitivity of growth rate in the species comprising the community and their relative ability to exploit other resources such as TON, PO_4, light and DIC, which may be limiting.

It is evident from Figs. 56-63 that a great deal of variation in species abundance also exists along canonical axis 2, which is closely related to the variables % Forest, A_{250}, Stream Order, Area or TON, in some analyses.

It is possible that the distribution of cyanophytes may be influenced by the TON concentration. In Figs. 60-63 *Phormidium* 8 µm and *Phormidium* 13 µm are closely associated with the head of the TON arrow (high concentration), while *Scytonema, Stigonema* and *Tolypothrix* lie towards to other end (low TON). The latter three species form heterocysts while *Phormidium* spp. do not.

No other clear candidates for relationships between particular species and environmental variables can be seen in these analyses. A larger data set would be necessary to enable the effects of multiple environmental variables to be disentangled.

Canonical Correspondence Analysis is thus seen to give important and consistent information about the influence of environmental variables on the distribution of filamentous algal taxa. The most important environmental gradient is one associated with acidification. The ultimate test of the validity of the relationship revealed however is whether the information can be used to reconstruct site pH values from the species data (calibration).

4.7.3.5 Calibration using CANOCO

Inferring environmental values from species data (calibration) by CANOCO is described by Ter Braak (1988). An analysis is carried out using a calibration set of data, with both species and environmental data included. It is necessary only to include one environmental variable, the 'variable-of-interest', in this process. The samples for which environmental variable values are to be inferred are included in the species data only, and are thus passive samples in the analysis.

Mean values of pH are inferred from the CANOCO output of the analysis according to the formula:-

$$\text{inferred pH} = Z + SX/C$$

where Z is the mean and S is the standard deviation of pH values for the calibration sites, C is the canonical coefficient for pH, and X is the sample score on axis 1, which are all given in the CANOCO output (Ter Braak, 1988).

This procedure has been carried out using Matrix C as the calibration data set and species data collected on four occasions in the Loch Dee catchment, Galloway, and on two occasions in the Loch Rannoch area. The values of pH inferred for the original data set (Matrix C, abundance data) have been plotted against the measured values in Fig. 67a, and the inferred and measured values for the Galloway and Rannoch data in Fig. 67b. The regression shown in Fig. 67a, lies very close to the ideal, which is the diagonal in this graph. However the Galloway and Rannoch data yield a much less significant regression, and the slope is well removed from the ideal.

A similar analysis has been carried out on the two halves of of the L. Ard data set, using matrix B as the calibration set (Figs. 68, 69). Using abundance data the autoregression (Fig. 68a) of the matrix B data is poorer than that obtained with matrix C, but with pooled p/a data an almost identical fit is found. The inferred pH values in matrix A however bear a less close relationship to measured values, although they are closer than the fit obtained with the Galloway/Rannoch data.

A further attempt to use CCA calibration was carried out using matrix C p/a data as the calibration set for matrix D (L. Ard, 1985) data. The fit is very poor.

The distribution of points in Figs. 68b and 69b is curious in that a second-order regression line can be fitted very closely (not shown). The significance of this is uncertain but it appears that the actual pH of the most acidic sites is somewhat lower than that predicted from the floral data. One possible reason is that the species present at these sites are resistant to pH excursions below those values found in Matrix B.

The poor fit obtained with matrix D and Galloway/Rannoch data is most probably due to the low number of samples which make them up, and the smaller species list compiled for matrix D (Kinross, 1985).

Dixit *et al* (1989) used CCA ordination in a study of chrysophyte species assemblages in lakes in Sudbury, Canada, a region heavily impacted by acidification due to a large ore smelter. Their study included seventy two lakes, with measured pH values between approximately 4.0 and 8.0. Each lake contributed one sediment core for the study of chrysophyte scales and one integrated water sample. Twenty-one taxa were utilized, ignoring rare species. This number of samples and species is suited to CANOCO, enabling up to seventy different environmental variables (in theory) to be considered. In fact they incorporated 23 variables. The most important were found to be pH and Cu^{2+}, and the possible use of chrysophyte scales for inferring pH was investigated by dividing their 72 lakes into two sets, a calibration set of 48 and a passive set of 24. Their results for inferred pH were highly correlated ($r = 0.9$) with measured pH, and better than those obtained by multiple regression.

These results are encouraging for those contemplating the use of CCA ordination and calibration. In comparing the results of Dixit *et al* (1989) with those presented here, the following points must be considered.

1. Each lake was sampled only once for chemical determinands. Notwithstanding the inherent stability of a large water body, variations in water quality may occur. It would be expected that use of a mean value for these parameters would improve the predictive value of the ordination.

2. The relative contribution of chrysophyte taxa was obtained by counting 500 scales. This is a more certain and accurate method of obtaining species abundance data than estimation of relative abundance.
3. Only one sediment sample was taken for the enumeration of scales, but the planktonic chrysophytes would be expected to be well distributed around the lake, such that a representative sample would sediment in the deepest part, where the samples were taken. This is not necessarily the case with diatoms, where benthic and planktonic forms may contribute different proportions depending where the samples are taken (Anderson, 1989; Anderson and Renberg, 1990).

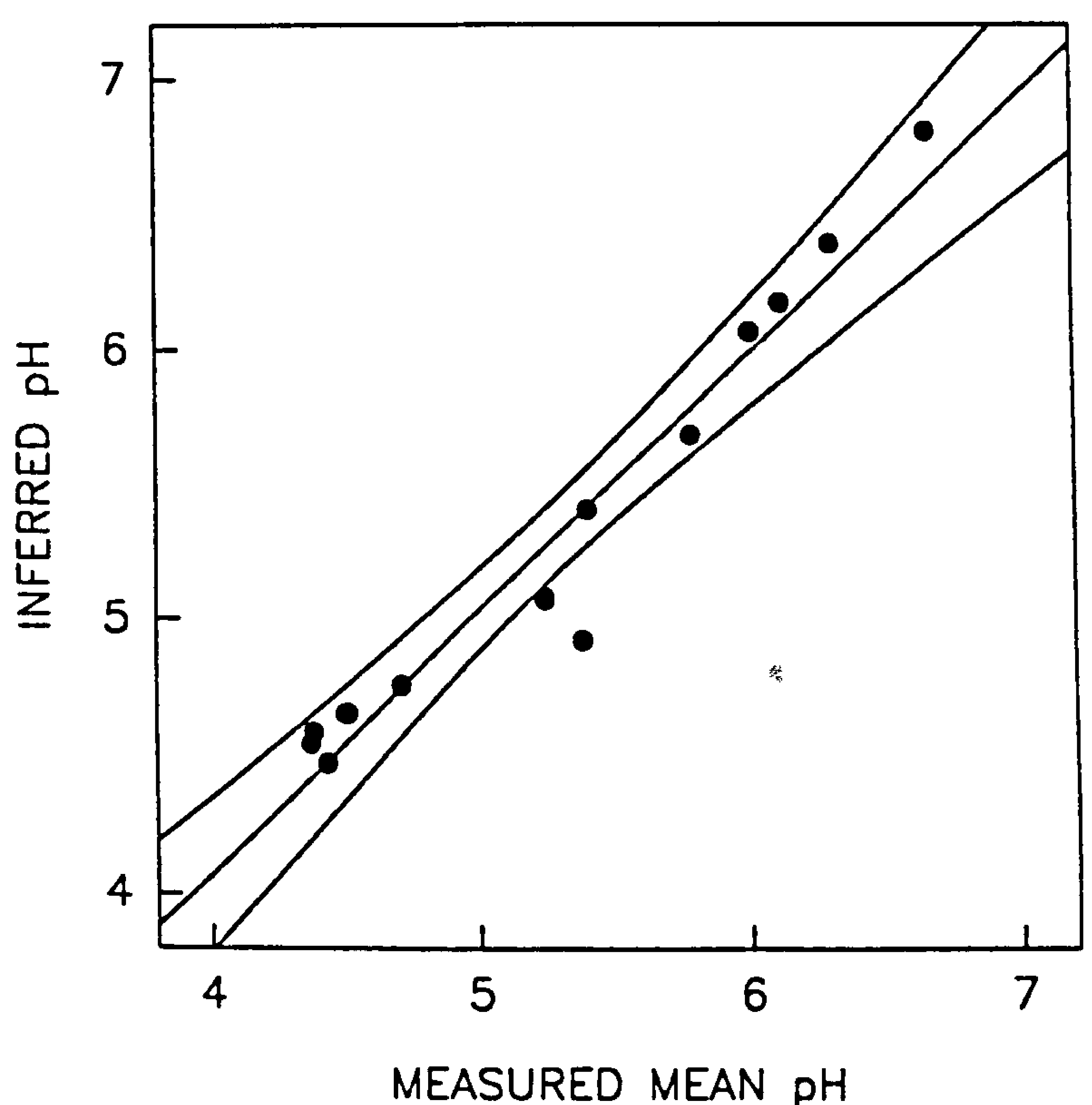
Taken together these factors may explain the high predictive value of chrysophyte assemblages in comparison with filamentous benthic algae.

Another reason might be the larger number of sites (lakes) in the Dixit *et al* (1989) study, giving the ability to include a larger number of environmental variables as well as a better coverage of the range of each variable.

These attempts to use calibration by CANOCO and apply it to species data on 1985 Loch Ard data and data from Galloway and Rannoch sites, show that when the number of samples used to make up the data matrix is less than about 15, a wide scatter of points is obtained so that the inferred pH values are unreliable (not significant at 5%). This reemphasizes the findings of Normal Association Analysis that a broadly based data set must be used. Until such data sets are available from other areas, the L. Ard data cannot be used as a calibration set for inferring environmental pH.

FIG.67: REGRESSION OF INFERRED pH VALUES OF SITES (USING CANOCO) ON MEAN MEASURED pH. (ABUNDANCE DATA) (99% C.I.)

(a) Loch Ard sites, Matrix C data.(n=35)



(b) Galloway and Rannoch sites.(n=4,2)

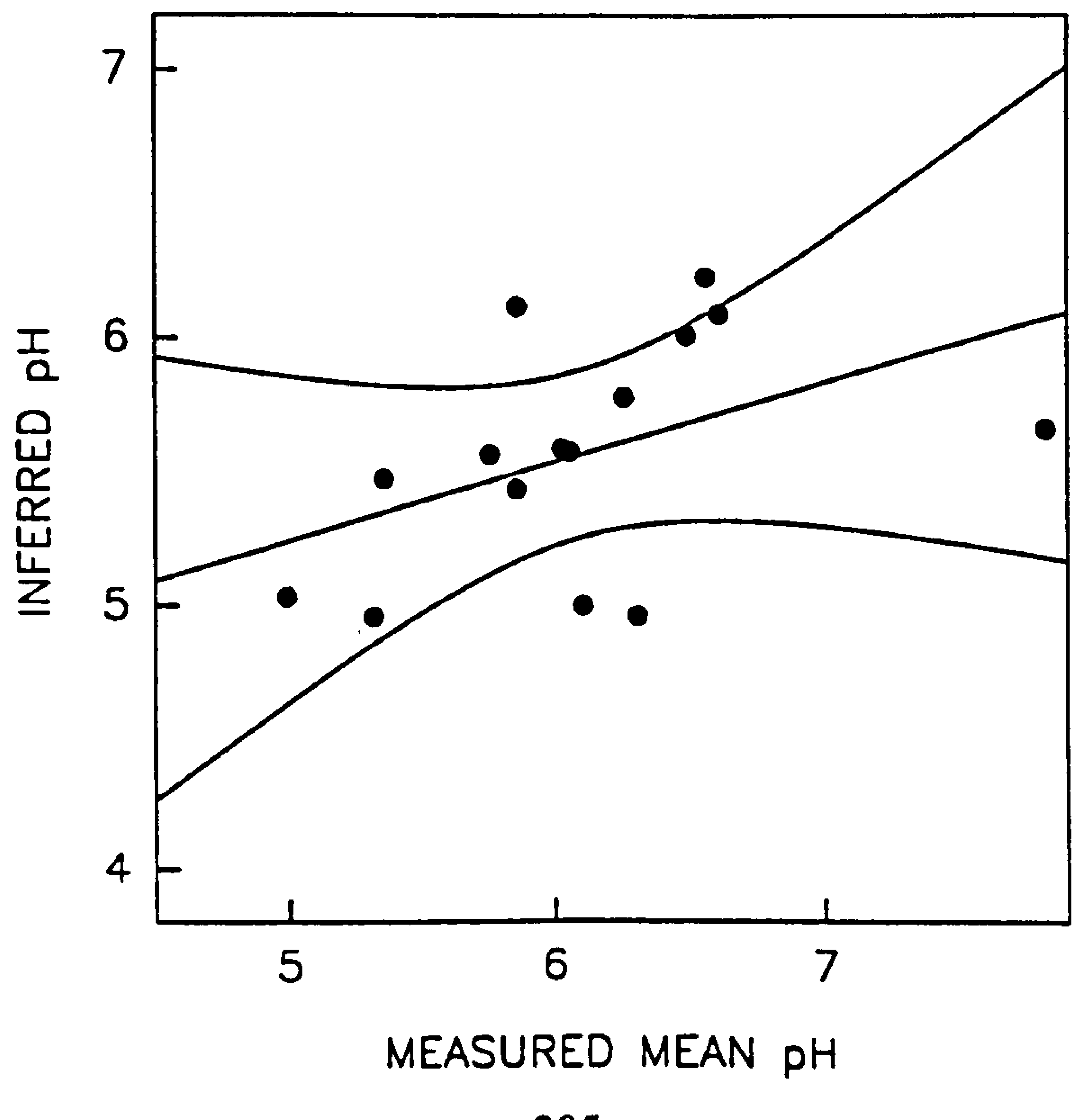
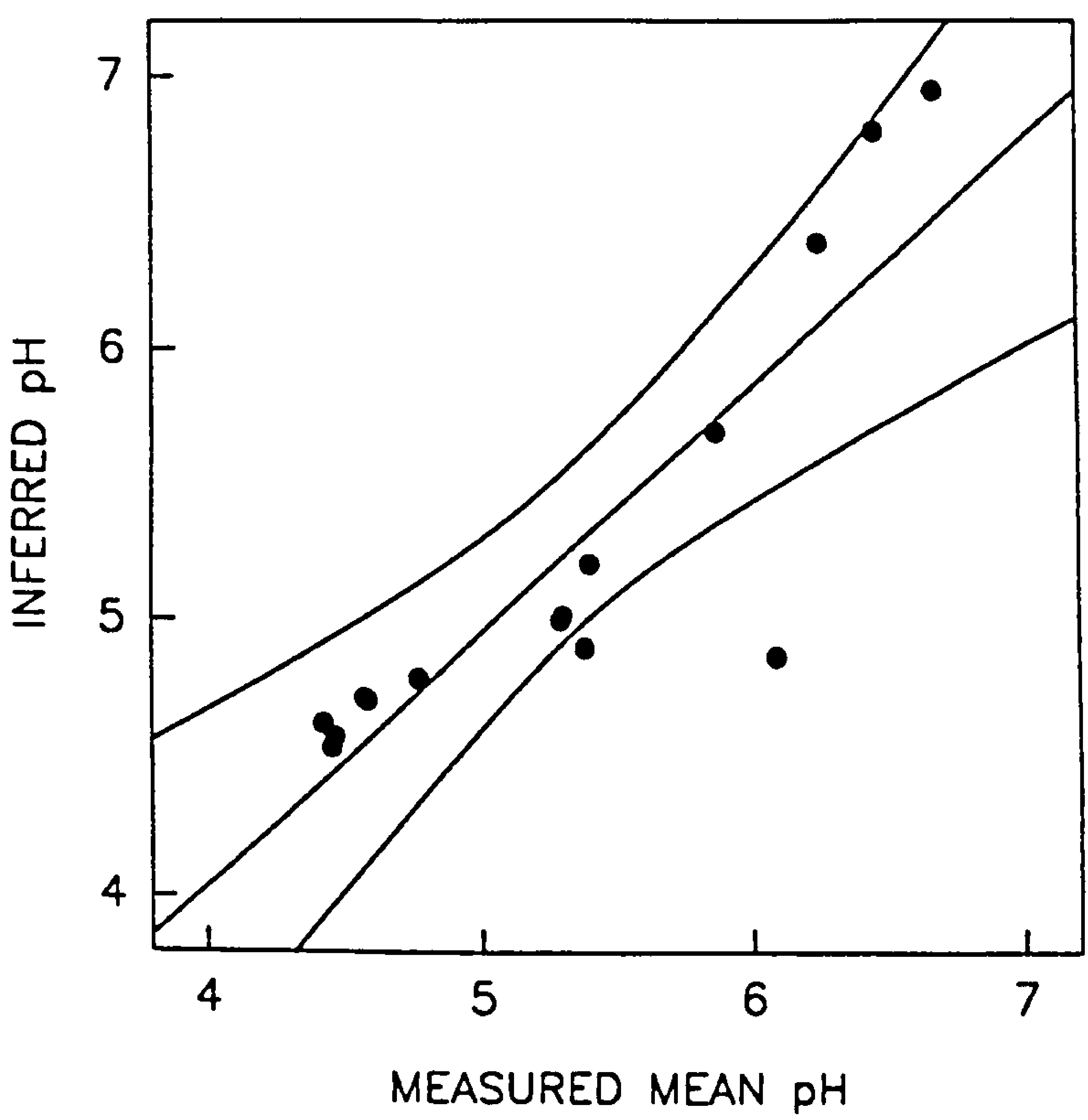


FIG.68: REGRESSION OF INFERRED pH VALUES OF SITES (USING CANOCO) ON MEAN MEASURED pH. ABUNDANCE DATA (99% C.I.)

(a) L.Ard sites, Matrix B data (n=18)



(b) Matrix A data.(n=17) pH inferred from matrix B data

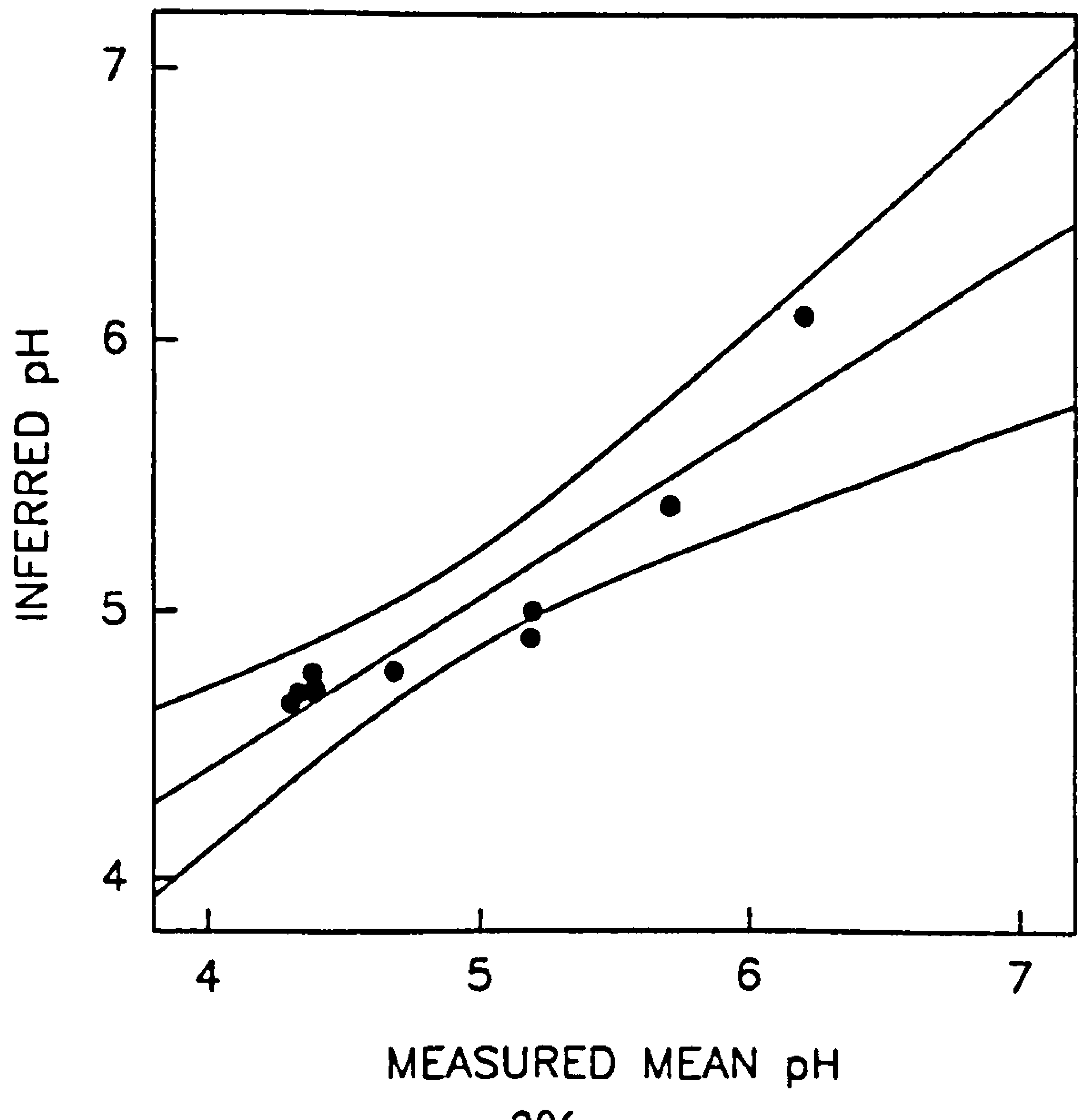
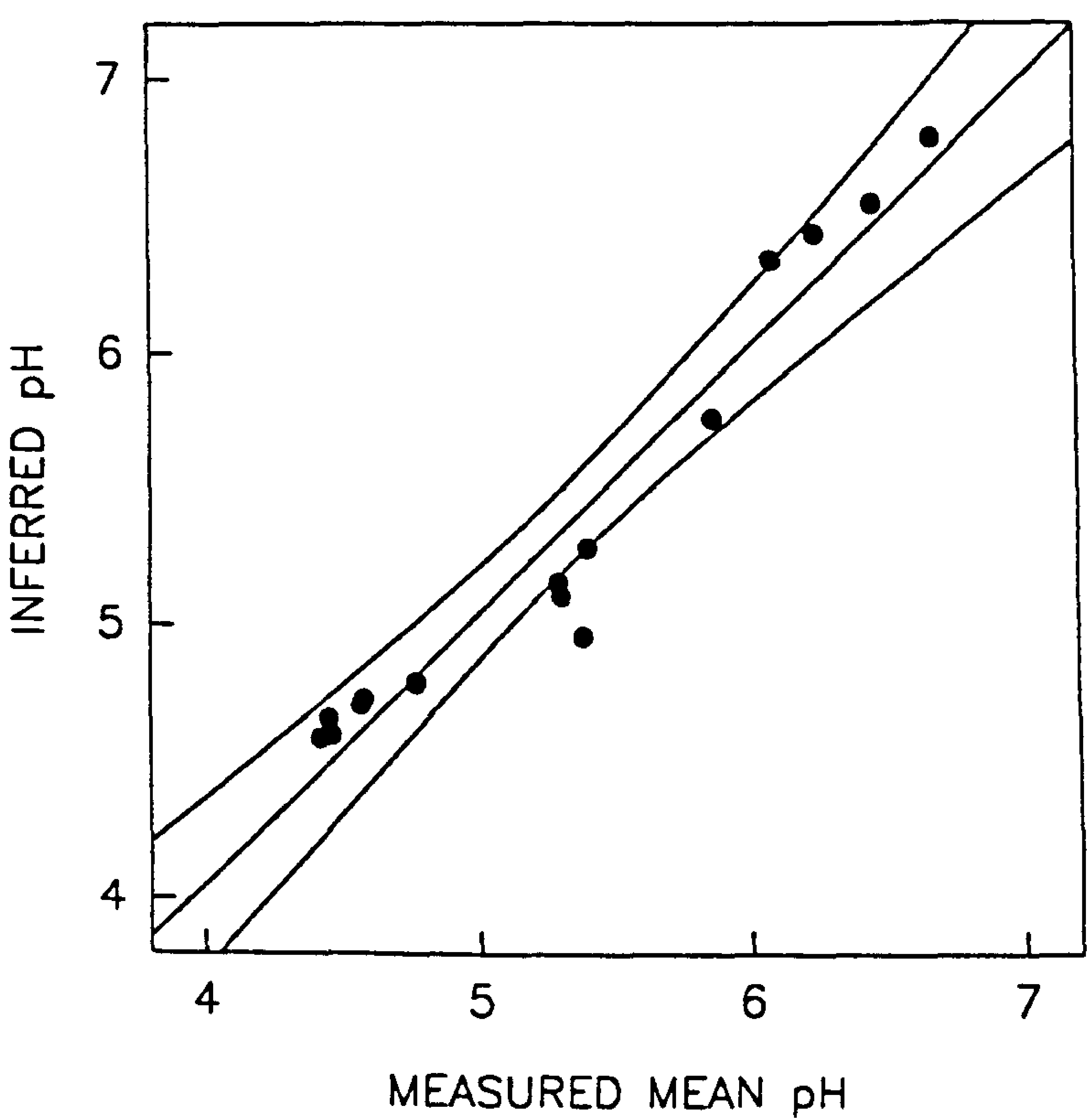


FIG.69: REGRESSION OF INFERRED pH VALUES OF SITES (USING CANOCO) ON MEAN MEASURED pH. POOLED P/A DATA (99% C.I.)

(a) Loch Ard sites, Matrix B data. (n=18)



(b) Matrix A data (n=17) pH inferred from Matrix B

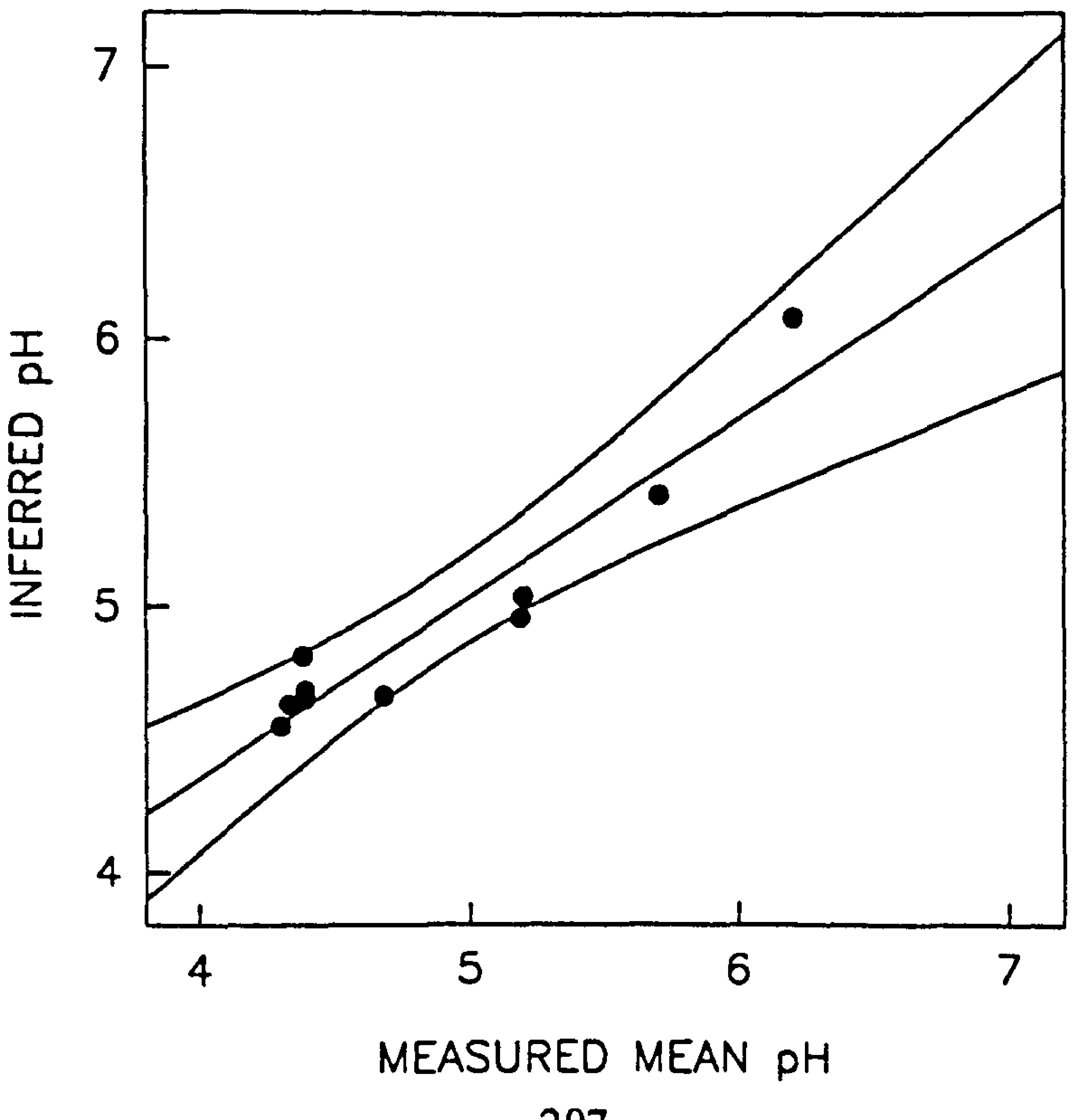
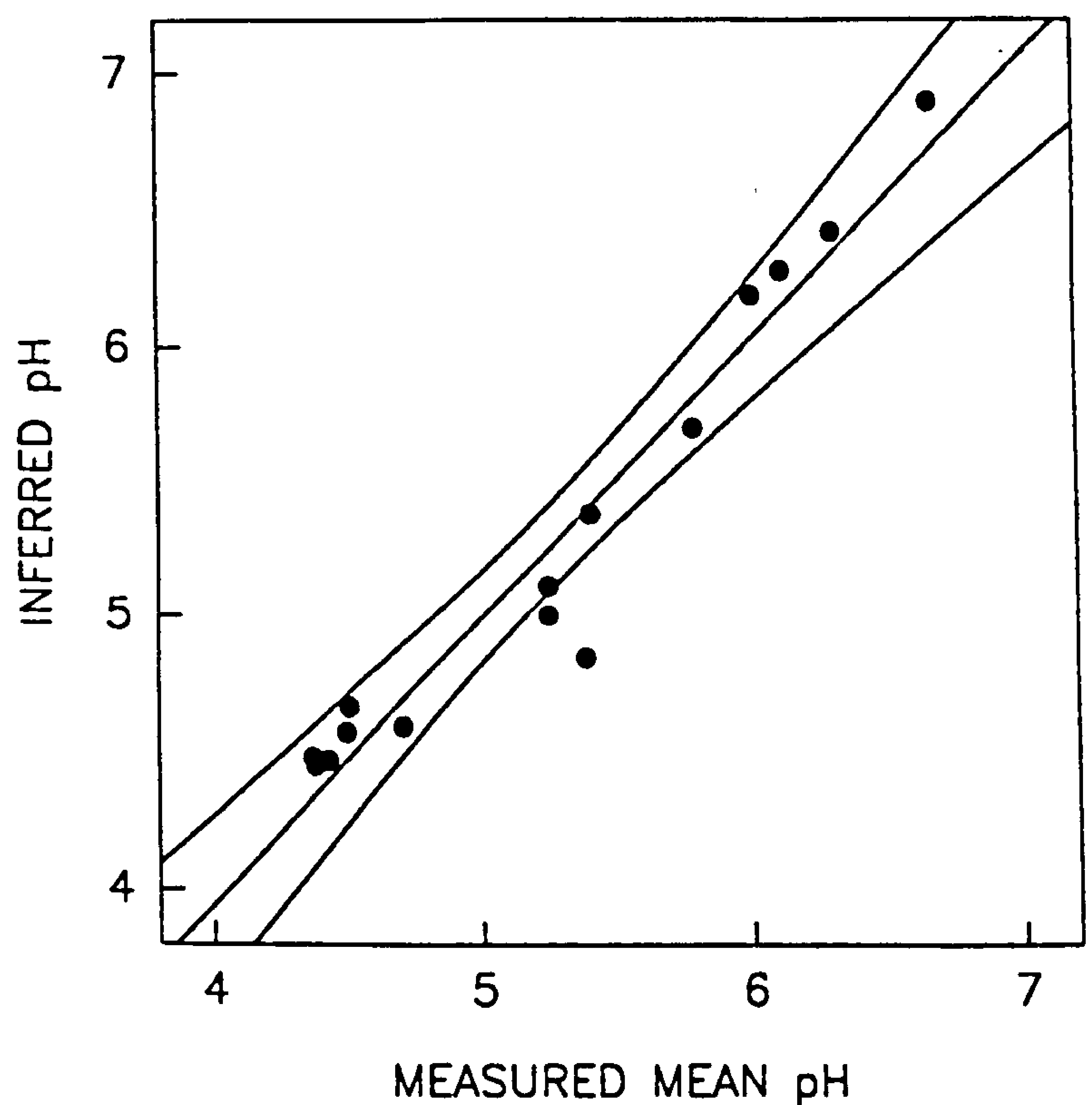
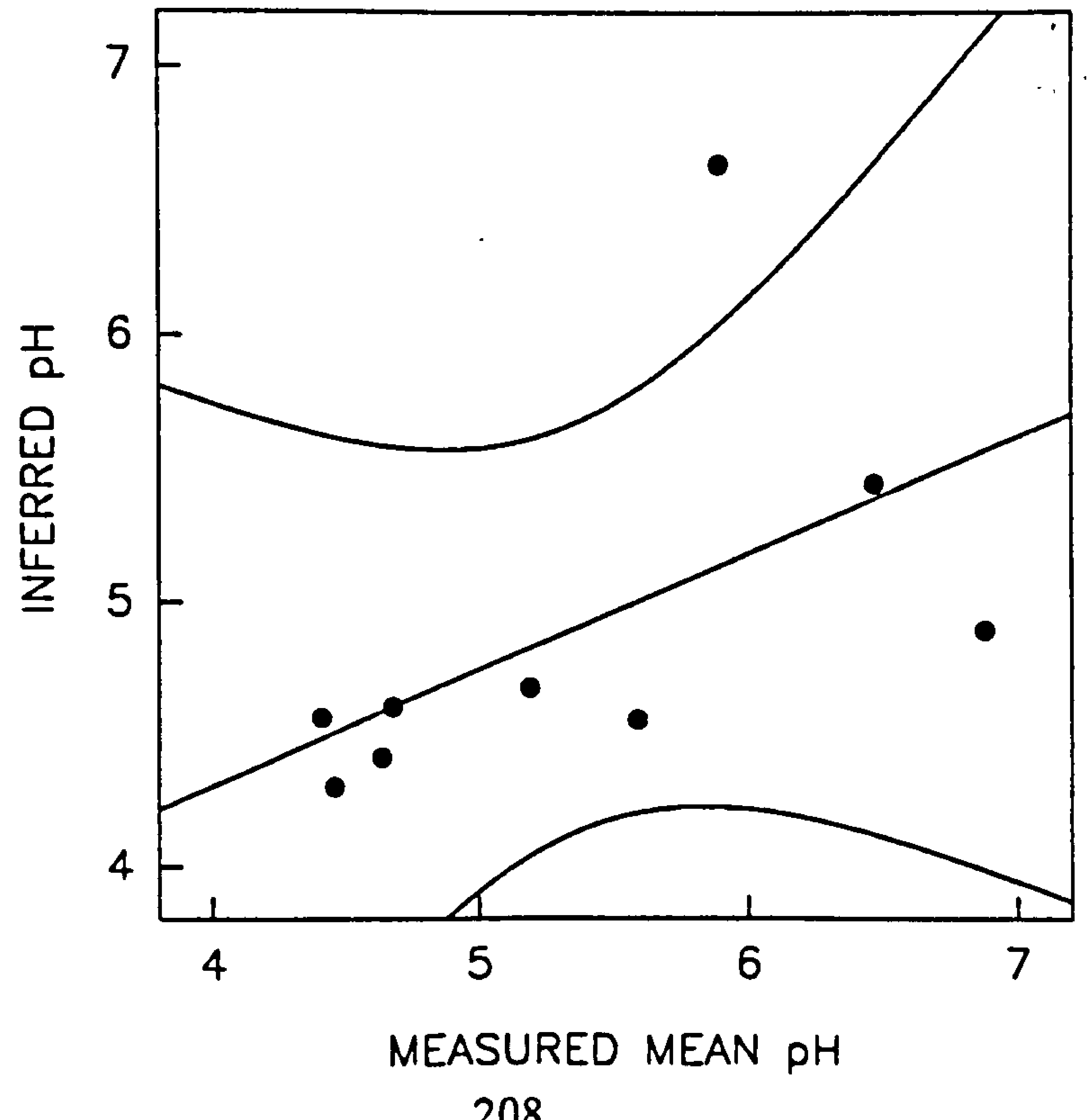


FIG.70: REGRESSION OF INFERRED pH VALUES OF SITES (USING CANOCO) ON MEAN MEASURED pH. POOLED P/A DATA (99% C.I.)

(a) Loch Ard sites, Matrix C, (n=35)



(b) L.Ard sites, Matrix D,(n=10) pH inferred from C



4.8 ALGAL PRODUCTION

4.8.1 Methods for measuring production rates

In the measurement of production rates both in the field and laboratory, several methods are possible. The assimilation of carbon may be measured as uptake of ^{14}C from bicarbonate, from flux of dissolved inorganic carbon (Turner *et al*, 1983) or as changes in pH consequent on the uptake of CO_2 from the water (Wright and Mills, 1967). Production of O_2 can be measured, either by Winkler assay or by use of a dissolved oxygen meter, by comparing values upstream and downstream, or diurnal changes at one station (Odum, 1956). Rate of biomass increase may also be measured as change in photosynthetic pigment yield or AFDW per unit area over a time interval. Unfortunately all these methods have limitations which render them less useful in environments such as the L. Ard streams.

The ^{14}C methodology may be used in the laboratory or in respiration chambers in the field (Bott *et al*, 1978; Duthie and Hamilton, 1983; Turner *et al*, 1983; Mulholland *et al*, 1986) but it relies on the presence of bicarbonate ion in the water. At the pH values prevalent in many of the L. Ard streams (pH 4-5) the bicarbonate would be dissociated and might be lost as CO_2 (Schindler *et al*, 1972).

Diurnal changes in pH as a consequence of CO_2 uptake during the day have been noted (Morgan, 1985; Wright and Mills, 1967. This phenomenon has been detected in streams in Galloway which are susceptible to acidification, but only at pH values in excess of 6.0 (Solway River Purification Board, unpublished observations), where the buffering is largely due to carbonate/bicarbonate (Ulrich, 1983 b). The equations for calculating carbon uptake from pH depend upon there being no other buffer system operating (Kelly *et al*, 1983). Morgan (1985) found the pH in an acidic lake raised from about 4.5 to 8 by photosynthetic activity enhanced by cultural eutrophication. Nutrient-poor running waters may be too well aerated for such pH increases to occur, and may in addition have a considerable accrual of CO_2 from groundwater percolation (Seip and Tollan, 1978, Reuss and Johnson, 1985).

Methods of estimating production from upstream/downstream oxygen concentration have been devised (Odum, 1956, Kelly *et al*, 1983) but these are again workable only in situations where the flow conditions are such that the exchange of gases between the water and the atmosphere can be calculated (which is not the case in rapidly flowing, shallow turbulent streams (McConnell and Sigler, 1959)) or in situations where exchange can be controlled or eliminated (Edelmann and Wuhrmann, 1978; Tease *et al*, 1983, Tease and Coler, 1984, Gallegos and Scheibe, 1986).

Measurement of changes in DIC have been utilized in acidified lakes (Turner *et al*, 1987) but would be more difficult to apply in running water, for the reasons just given.

4.8.2 Biomass

In the absence of direct measurements of primary production, the standing crop of primary producers may be taken as an indicator of production rate in this field.

An increased algal standing crop has been reported in acidified water (Hendrey, 1976; Muller, 1980; Mulholland *et al*, 1986). The cause has been questioned. Stokes (1986) lists four possibilities, decreased grazing by acid-sensitive invertebrates being one. Removal of grazing pressure by factors other than acidification has been shown to result in an increased periphyton biomass (Eichenberger and Schlatter, 1978; McAuliffe, 1984; Yasuno *et al*, 1985; Jacoby, 1987; Feminella, 1989; Winterbourn, 1990). Decreased decomposition as a result of acidification is also proposed as a cause of the reported biomass increase. The other two possibilities are a shift towards dominance by acidobiontic algal species and a decrease in competition between species.

If such an increase upon acidification is real then productivity cannot be compared between acidified and non-acidified streams. Biomass in acidified streams will reflect the primary productivity more closely, without the necessity of considering loss factors.

The relationship between biomass and environmental variables has been investigated by calculating Pearson's correlation coefficient, r, between variables and different measures of biomass. This has been carried out using both mean values for each sampling site, when the appropriate significance level is 5%, and individual values from all sites on all occasions, when the appropriate significance level is 0.1-1% (Tables 17, 19). The results show a high degree of variability but tend to support the view that acidification does not result in an increase in algal biomass, indeed they suggest that the opposite is the case. The reasons for this however are uncertain.

4.8.3 Production rates in laboratory studies

Estimation of rate of increase of biomass in the field is complicated by sampling difficulties, in particular the difficulty of providing replicates in view of the patchy distribution of benthic algae (Butcher, 1941; De Nicola and McIntyre, 1990) and of the high probability of loss of biomass and/or substrates with changes in discharge between visits.

This is not a problem in the laboratory but there remains the difficulty of obtaining an even colonization of artificial substrates such that the reproducibility between replicates is acceptable (Meier *et al*, 1983). This was found in preliminary trials to be too serious a difficulty to make this method feasible.

Instead the method of Rosemarin (1983) was employed, in which the growth of individual filaments is followed over a time course by direct counting of cell number. This has the advantage of allowing the use of mixed cultures, allowing a great deal of information to be gathered in one experiment, but with the limitation that only fairly low filament densities and short filament lengths can be used. Thus the growth rates obtained may not be relevant under conditions of high biomass.

Changes in average cell length may lead to inaccuracies in the growth rates obtained. Some species appear to show synchrony of cell division through several generations; *Hormidium subtile* and *Geminella* 8 µm. are most frequently found in filaments of 4, 8, 16, 32 or 64 cells under the conditions employed. Thus it is necessary to obtain counts from a large number of filaments over several time intervals to even out the consequence of a sudden doubling of cell number in a given filament. Growth might also be measured by increase in the length of filaments; however this could not be done directly under the microscope due to the tortuous nature of many of the filaments, which also rules out photography of a microscope field as a means of obtaining material for measurement, as only a small proportion of the filaments will be in focus at any time. The use of a drawing tube might make measurement of filament length possible, but at the cost of a reduced number of possible observations.

Despite possible inaccuracies or limitations of the cell-counting method, it does have some advantage over short term measures of production such as are obtained by measurement of ^{14}C uptake. Knoechel and De Noyelles (1980) found that short-term bioassays tend to overestimate the accrual of biomass because they neglect possible extracellular release fo fixed carbon. On the other hand ^{14}C labelling followed by track autoradiography (Knoechel and Kalff, 1976) offers the opportunity of simultaneous measurement of uptake rates of different species in mixed culture if the culture conditions, particularly pH, permit its use.

For production measurement by ^{14}C uptake, or by pH measurement or O_2 production, the algae must be enclosed in apparatus which restricts exchange with the atmosphere while permitting a current regime to that obtained in the open channels to be maintained. An attempt was made to grow algae attached to a slide (removed from a channel after a growth rate experiment) in a chemostat jar with a stirrer and O_2 electrode. It was found that the condition of the algae deteriorated steadily so that repetition of O_2 measurements could not be relied upon. The culture conditions were evidently not suitable in the long term, so short term measures carried out under these conditions could not have been related to the growth rate measurements carried out over 1-2 weeks. Further work on this problem would be necessary to enable measurements of O_2, DIC or pH to be utilized.

If it can be shown that the short-term measurements are reliable and can be related to long-term growth rates, then the micro-methods described by Brammer and Hogberg (1980), Wetzel *et al* (1984), and Brammer (1988), utilizing samples of plant material enclosed in a Cartesian diver, may permit rapid assessments to be made of the effects of environmental variables on photosynthesis in different species.

4.7.4 Growth rate measurements

The results obtained from the statistical analyses of field data all suggest a relationship between community structure and acidification-related environmental variables. However there is no way of determining from these which of the many intercorrelated variables are actually responsible for the observed species distribution.

The thesis that pH and/or Al are the determining factors was investigated by experimental manipulation of the growth conditions of algal isolates from natural populations. The temperature, light regime, current velocity and DIC levels were held constant, allowing aspects of water chemistry to be independently manipulated.

In the first series of experiments pH was adjusted by addition of dilute H_2SO_4. Additional sulphate was thus added, raising the initial concentration of 193 $\mu eq\ l^{-1}$ to between 270 $\mu eq\ l^{-1}$ and 350 $\mu eq\ l^{-1}$, depending upon the target pH of the medium (6.0 to 4.0 respectively). The initial and final values of TON, PO_4, and, for run 3, Aluminium concentrations are shown in Table 22.

In the second series of experiments, the pH was held constant at 5.5 and the proportions of Al and Si were varied. Treatment A consisted of 'Corrie' medium adjusted to pH 5.5, B was a modified medium with Si reduced from 6.0 to 5.5 µM in order to test the thesis that the high [Si] in 'Corrie' protects against the effects of Al, and treatment C had the concentration of Al raised from 40 to 146 µg l^{-1} in addition to the decreased Si concentration. The actual concentrations of Al, Si and the major nutrients N and P were measured at the beginning and end of the experiments. Two experimental runs were performed, using different species. In each run, three pairs of channels were used with the three treatments.

Comparison can also be made for some species with growth measurements made previously in the acidic medium B9, with a pH of 4.6. Si and Al concentrations were 27 µM and 146 µg l^{-1} respectively. N and P concentrations in B9 differ markedly from Corrie; B9 is N-limited whereas Corrie is P-limited. Differences in the ability of some species to grow in these media may be due to the complexation of phosphate with Al in B9.

The reduction of Si concentration is accompanied by a decrease in the concentration of Na^+ from 175 to 168 µeq l^{-1} (by calculation) but readjustment of pH by addition of NaOH causes the final measured Na concentration to increase from 220 µeq l^{-1} in A to 240 in B and 400 in C. Similarly, increasing Al concentration results in an increase in Cl^- from 240 to 252 µeq l^{-1}. As a consequence of pH adjustment the sulphate concentration in the pH 4 channel is 80 µeq l^{-1} higher than the 270 µeq l^{-1} in the pH 6 channel. Table 24 shows the initial and final values of TON, PO_4, Si and Al concentration in these experiments.

The changes in Na^+, SO_4^{2-} and Cl^- which accompany the experimental treatments cannot be formally excluded from a role in influencing growth rates and species distribution, but are probably of minor or secondary importance in view of the relatively high starting concentrations of these ions.

Growth rates of algae are potentially affected by light, temperature, nutrients and the supply of inorganic carbon. These factors are interrelated. If growth is limited by the availability of one resource, the others may have their effects modified or even negated (Raven and Geider, 1988). This has led to reports of different resources limiting productivity in aquatic systems. Light levels have been implicated in an increased biomass in acidified lakes, supposedly due to greater light penetration as the humic waters become clearer (Effler *et al*, 1985; Shearer *et al*, 1989; O'Grady and Brown, 1989).

In shallow, lotic environments with similar water chemistry, light penetration may not be limiting but shading may cause light limitation of growth (De Nicola and McIntyre, 1990a).

Measured levels of PAR at some sites were as low as 40% of those at open sites, while other unmeasured locations on some streams would undoubtedly be even lower due to shading by vegetation. However the levels of PAR supplied to the growth channels was approximately 25 µmoles $m^{-2}s^{-1}$ during the pH experiments and 40 µmoles $m^{-2}s^{-1}$ during the Al/Si experiments, approximately 3% and 6% of normal daylight levels (summer). At these levels some algae might be expected to be light limited (Talling, 1957).

Different species associations occur under different insolation conditions (Steinman and McIntyre, 1986; O'Grady and Brown, 1989), implying a differential effect of light on the growth of different species.

Differences in current velocity may also affect growth rates, taxonomic composition and biomass accrual, although these effects are secondary to any differences in nutrient concentration or PAR (McIntyre, 1968; Horner and Welch, 1981; Horner *et al*, 1983, 1990; De Nicola and McIntyre, 1990a, b).

Current velocity in the channels varies between 11 and 25 $cm.s^{-1}$, depending on biomass, compared with values between 0 and approximately 100 $cm.s^{-1}$ in the field. Current velocities between about 15 and 50 $cm.s^{-1}$ appear to enhance the development of periphytic biomass (Horner and Welch, 1981; Horner *et al*, 1983). This may be due in part to increased exchange of nutrients and waste materials, compared to the rates possible solely by diffusion across the boundary layer (Patrick, 1968; Bothwell, 1989).

Furthermore if the water surface is disturbed the rate of dissolution of CO_2 from the atmosphere will be increased. In acidic waters the amount of dissolved CO_2 may limit primary productivity (Wetzel *et al*, 1984; Mulholland *et al*, 1986; Turner *et al*, 1987). An increased supply of DIC has been found to enhance primary productivity under such circumstances (McIntyre and Phinney, 1965; Dickman, 1973).

The design of the channels is such that an adequate supply of DIC should be available; since air is pumped into the water flow at the head of the channel it is expected that the water in the channel will be saturated with respect to atmospheric CO_2. However it was not possible to check DIC levels in the different channels and hence any differential effect of pH on the availability of DIC is unknown. Likewise in the field, the levels of DIC available are unknown. Lerman and Stumm (1989) report that lakes are near equilibrium with atmospheric CO_2. Flowing water should be even closer to equilibrium (although periphytic algae in a shallow body of water may disturb this). The available DIC is therefore largely dependent on the alkalinity of the water (Lerman and Stumm, 1989) and should therefore be less at lower pH values. Below pH 5.6 essentially all DIC is in the form of dissolved CO_2 (Stumm and Morgan, 1981).

At low light levels (20-90 μmoles $m^{-2}s^{-1}$ PAR) the primary productivity rates of periphyton are similar at different current velocities (De Nicola and McIntyre, 1990). It appears likely therefore that light may be a limiting resource in the channels and that current is immaterial. However if nutrients are limiting, e.g. available PO_4 less than 0.1 μg l^{-1} (0.01 $\mu eq.l^{-1}$), PAR changes may have no effect on growth rates (Bothwell, 1988). Bothwell (1988) also found that when PO_4 was not limiting temperature had a strong influence on growth rates.

Coesel and Wardenaar (1990) found that species of planktonic desmid were temperature-limited for growth below 15°C, but at higher temperatures light became the limiting factor (levels tested were between 8 and 150 μmoles $m^{-2}s^{-1}$). Therefore at 10°C, the standard temperature used in the experiments described in this work, light may not actually be limiting, even at 25 $\mu moles.m^{-2}s^{-1}$.

10°C is a realistic temperature for growth of the algae studied as field temperatures were between 5 and 15°C for most of the year, going as low as 1°C only in low-flow conditions in winter, and reaching 22°C during similar conditions in strong summer sunlight. However a constant temperature is not a realistic environmental condition. In the field, temperature may vary by up to 8°C diurnally, which differentially affects the rates of photosynthesis and respiration (Round, 1973; Davison, 1991).

Kelly *et al* (1983) found no effect of temperature on the rate of photosynthesis in the field. Light harvesting is not sensitive to temperature (Raven and Geider, 1988) but growth rate may be affected, each species having an optimum temperature for growth (Patrick, 1968; Davison, 1991). At temperatures above and below this, the net photosynthetic rate declines. Temperature may influence the availability of DIC at pH values below 5.6 due to its effect on the solubility of gases.

In comparison with medium B9, Corrie medium is strongly PO_4^{3-} limited, hence the transfer of old cultures into B9 medium to encourage zoosporogenesis (cf. Reynolds, 1950). Nevertheless, at 50 $\mu g\ l^{-1}$ PO_4-P, the concentration is high enough that differences in current velocity may be more important, because of diffusional constraints (Horner and Welch, 1981).

Although the same amount of PO_4^{3-} was supplied in all the variations of Corrie medium used, its availability may be affected by complexation with metals, principally Al and Fe (Minzoni, 1984; Nalewajko and Paul, 1985; Nalewajko and O'Mahony, 1988). Liberation of metals and phosphate from these complexes may occur if the pH is lowered (Nalewajko and O'Mahony, 1988).

Assay of nutrients, Al (and Si) was carried out on the channel media at the beginning and end of growth experiments, but the samples were not filtered prior to assay so that particulate complexes would not have been excluded. In some analyses levels of Al were found to rise with time implying a slow release from complexation (Tables 22, 24) PO_4^{3-} concentrations also rose with time in some media which had not yet been inoculated (data not shown), but where algae were present uptake might mask any such release. Precipitates could remain undisturbed at the bottom of the medium bucket despite the presence of the circulation pump, and would therefore only slowly release their component ions into solution.

Nalewajko and O'Mahony (1988) found that phosphate released from complexes in natural water in response to acid shock emanate principally from particles greater than 0.45 µm in size, therefore filtration is necessary if it is desired to discriminate between total and soluble PO_4^{3-} in field samples as well as in culture media.

However simple measurement of dissolved phosphate concentration is not enough to indicate whether algal growth in a body of water is limited by P availability (Nyholm and Lyngby, 1988).

Differences in nutrient availability may lead to differences in taxonomic composition (Lehman, 1976; Evenson *et al*, 1981; Carrick and Lowe, 1989; Bothwell, 1989). Unfortunately phosphate concentrations in Corrie medium and in many field samples, are at the limits of detectability by the assay method used. Therefore it is uncertain whether any variation in PO_4^{3-} availability with pH is occurring. Jansson *et al* (1986) found that lake water acidification resulted in lower PO_4^{3-} concentrations because of reduced input from the catchment, possibly owing to the formation of Al-PO_4 complexes in the soil. It is therefore not possible to state unequivocally what the effects of acidification will have on PO_4^{3-}concentrations in the field.

Of all these factors, DIC and PO_4^{3-} are the ones most likely to be influenced by the major variable, pH. Since the algae are allowed to settle on the slides in a phosphate-replete medium before transfer to the channels and many species are known to exhibit luxury uptake of nutrients, they are unlikely to be phosphate-starved at the outset, at least. Furthermore the cell density is very low at the stage when growth measurements can be made but will develop into a biomass many hundreds of times denser if allow to grow on, indicating no nutrient limitation. The mean daily increment of biomass in Runs 2 and 3 (Fig. 23) is similar, although the period of growth was twice as long in Run 3, again indicating that growth was not impaired by a depletion of nutrients up to at least 64 days.

While it is most probable that DIC levels are the same at all pH values below 5.6 and may be very similar at all pH values, these levels were not measured and therefore no definitive statement can be made. Therefore pH itself or an associated change in Al level is probably the major variable affecting growth rate in the pH experiments (runs 1-3).

Measured concentrations of aluminium in Run 3 fall short of the recipe values; in B9 the value is 52-56 compared to the target 146 $\mu g\ l^{-1}$ and at pH 4, the Corrie medium has only 14-23 instead of 40, while at higher pH it is less than 5 $\mu g\ l^{-1}$ (Table 22). The difference with pH is probably due to the dissociation of complexes.

In Runs 4 and 5, the Al/Si manipulation experiments, reducing the Si concentration (treatment B) results in no significant difference in growth rate from treatment A, but increasing the Al concentration to approximately 200 $\mu g\ l^{-1}$ (treatment C) causes a marked decrease in growth rate in all species tested except for *Hormidium subtile* and *Geminella* 8 µm.

In Run 4 the Al concentration in treatment C overshoots the target, while treatments A and B fall short of the target values. Because Si equivalence has been reduced below that of Al in treatment B, this cannot be ascribed to coprecipitation of Al with Si in treatment A. There is therefore effectively no difference between treatments A and B (apart from a slightly lower PO_4^{3-} concentration) and correspondingly no differences in growth rate are found.

Treatment A ought to have a similar concentration of Al-TM to Channel 5 (pH 5.5) in Run 3, while it clearly does not (Tables 22 and 24). Two factors which may be involved in this are the variability found in the results of the catechol violet assay for Al, for which no reason could be found, and the fact that different batches of 100 x concentrated medium were used in these experiments. These results emphasize the need for measurements to be made of major chemical variables such as Al in growth experiments, rather than a reliance on speciation calculations (Gensemer, 1990) and the need for alternative assays for low levels of Al and PO_4^{3-}.

In summary, the following factors may limit the applicability of the experimentally derived growth rate data to the field situation:

(a) Low PAR levels and constant light-dark cycle.

(b) Current velocity restricted to 10-20 $cm.s^{-1}$, compared to <10-100 $cm.s^{-1}$ in the field

(c) Constant temperature

(d) Unknown DIC concentration both in channels and in the field.

5. CONCLUSIONS

Filamentous algal communities present difficulties in quantitative sampling and enumeration because of their patchy distribution and filamentous nature. It has been demonstrated that these difficulties may be overcome by taking samples from all available habitat types within a sample site and scoring taxa for their relative contributions to the community. By taking multiple samples over a period, the data may be aggregated into a semi-quantitative form suitable for statistical treatments. As an alternative, to avoid possible bias in the estimation of relative abundance, the data may be expressed as presence or absence in each sample before being pooled.

Using the data in these forms, Inverse Association Analysis revealed the presence of distinct communities in streams with mean pH values towards the extremes of the range encountered. Normal Association Analysis and Cluster Analysis showed that sites can be divided, on the basis of their community structure, into groups which are related by their mean pH values. Canonical Correspondence Analysis confirms both of these findings and furthermore enables the ordinations of species and sites to be related directly to environmental variables. It is found that pH, acting as a marker for all acidification-related variables, has the greatest influence on the ordinations amongst those variables tested. The data set was subdivided chronologically for these analyses to test the internal consistency of the data. Substantially the same results were found, whether the whole data set, or the earlier or later half of the data set was used, in either the relative abundance or pooled presence/absence form.

The Canonical Correspondence Analysis program CANOCO (TerBraak, 1988) can also be used to infer field pH from community data. This was done for the earlier half of the data set, using the later half as a training set, and the results showed a close correspondence between the observed and predicted mean pH values. This analysis confirms that community structure may be used as an environmental indicator.

These statistical procedures cannot discriminate between the different variables which are closely correlated with pH as potential causes of the distribution of taxa. Therefore measurements were made of the growth rates of isolates of several algal taxa, to determine the independent influences of pH and aluminium. Measurement by direct counting of cell numbers in individual filaments is feasible only on attached, non-motile taxa at an early stage of growth on sparsely settled substrata. It was found that the optimum growth rate of the algae tested varied within the pH range tested (pH 4.0 to 6.0). Species characteristic of the more acidified sites (*Hormidium subtile* , *Mougeotia* 11µm., *Mougeotia* 17µm., and *Stigeoclonium* 5µm.) had higher growth rates (0.2-04 divisions/day) between pH 4.0 and 4.5 than species which are found in circumneutral sites (*Draparnaldia* sp. and *Stigeoclonium* 8µm.) which grow much more slowly or not at all at pH 4.0-4.5. *Oedogonium* species die below pH 4.5, but show growth rate optima at higher pH values which do not reflect their distribution in the field (Table 55), possibly because , in contrast to other species, they died rapidly in culture.

The effect of 200µg.l^{-1} of total aluminium, most of which was in the toxic labile form, was to cause

a rapid decline in growth rate in most species, apart from *Hormidium subtile*, *Geminella* 8µm. and one of two *Stigeoclonium* 5µm. isolates (in one replicate only). Taken together with the effects of pH on these species, the results explain why they have a similar ranking with respect to pH in the field. The growth rate of the *Stigeoclonium* isolate in that replicate which grew was the highest recorded,which may explain its place in the pH ranking, at a lower-pH position than *Hormidium* and *Geminella.*

In separate attempts to culture *Stigeoclonium* isolates for taxonomic studies, it was found that isolates from different streams were ecotypes with respect to pH, which may prejudice the use of these taxa for predictive purposes.

In contrast to some previous reports (Hendrey, 1976; Muller, 1980; Mulholland *et al*, 1986), but in accordance with other work on naturally acidified streams (Stokes, 1981; Marker and Willoughby, 1988), biomass measured in natural streams was not higher in low pH waters. In laboratory measurement it was found that the maximum biomass was developed around the pH optimum of the species present as revealed by the growth rate experiments. Differences in the ratio of chlorophyll-a to other biomass measures probably reflect the composition of the periphyton, with green algae contributing relatively more chlorophyll-a.

It can be concluded that differences in the species composition of populations at different pH in the experimental channels can be ascribed solely to species preference for pH or associated chemical factors, since no invertebrate grazing occurred, and heterotrophic activity can be presumed not to be reduced by low pH (in contrast to the reports by Hendrey (1976) and Muller (1980)) because biomass was not found to be greater at low pH. Thus while such factors undoubtedly play some role in determining biomass and species abundance in the field, it is not necessary to invoke them in order to explain why changes in taxonomic composition occur on acidification.

6. RECOMMENDATIONS FOR FUTURE WORK

Filamentous algae have not been extensively utilized as biological indicators, unlike diatoms (Patrick, 1973; Davis and Berge, 1980; Van Dam *et al.*, 1980; Flower and Battarbee, 1983; Batterbee, 1984; Charles, 1985; Dixit and Dickman, 1986; Charles and Smol, 1988; Keithan *et al*, 1988; Anderson, 1989; Holmes *et al*, 1989; Eloranta, 1990). Nevertheless they possess some advantages in the study of acidification, being present at pH values where diatoms are rare, although they do not permit palaeoecological surveys to be carried out.

In order for the best value to be made of indicator species, a good understanding of their autecology is required. This is rarely the case in the use of diatoms (see however Gensemer and Kilham, 1984) where the indicator value of a species is often determined solely from correlation analyses (Gensemer, 1990). In all algal groups therefore it would be advantageous to determine the growth rate of species under different environmental conditions.

The experiments described illustrate a method for determining growth rate in different environmental conditions which is suitable for some algal groups under some circumstances. The results

obtained show the effect of pH on growth and show that aluminium concentrations also differentially affect algae. The measurements require to be extended however for a more complete understanding of the autecology of these species. In particular different temperatures and higher intensities of PAR should be employed and growth measurements made at higher cell densities.

The contribution of diatoms has been largely ignored in these studies. Nevertheless the potential exists to examine the growth of non-motile species. The Germanium inhibition technique has been demonstrated to be a useful tool for examining the interaction of different algal groups and could be employed in the channel apparatus to permit the detailed analysis of the effects of competition between diatoms and green algae. Diatoms were present in many of the field samples and in some of the cultural studies also. Samples have been preserved for analysis, which could be related to future work.

In autecological studies serious consideration must be given to the possibility of ecotypic variation. Two problems arise. In the first, morphological variability is such that species cannot be positively identified, as in the genus *Stigeoclonium* (Cox and Bold, 1966). In the second, varieties of a species may occur which are specifically adapted to a particular environmental condition, so that this species cannot have any indicator value for that variable, a situation which also arises with *Stigeoclonium* species (Francke and TenCate, 1980; Francke, 1982; Francke and Rhebergen, 1982).

Under these circumstances it is extremely important to compare the autecology of different isolates of a 'species', to determine whether such ecotypes exist. Polymorphisms are common in natural populations and it is important to know whether the indicator value is compromised. Clonal, unialgal cultures should be used as inocula, even if mixed species are used in growth measurements.

A third type of variability can arise which however may not pose a problem in practice. If morphological variation in a character occurs in response to an environmental variable, that character may be utilized as an indicator. Thus if two types can be distinguished, one always associated with, say, pH below 5.0, the two types may simply be scored as separate taxa for indicator purposes.

The method of relating species occurrence in the field to environmental variables employed in this study was determined by the particular sampling problems associated with filamentous algae. In essence it involved taking a large number of separate samples, spread out in time in order to build up a frequency table for the different taxa. Conventionally, where counts of species abundance can be carried out, the procedure would be to take one large sample (albeit with several subsamples) from each site and to perform a detailed count on it (e.g. Dixit *et al*, 1989). Taking samples across a period of time has advantages in enabling difficult specimens to be identified (Hillebrand, 1973) but has the obvious drawback in being expensive in time. It might be possible to reduce the time required to a few consecutive weeks or months by taking multiple simultaneous samples, perhaps using a larger reach of a stream to maximise the chances of including rarer species and obtaining multiple records of the more common ones.

The use of CANOCO for analysing and interpreting species-environment interactions is likely to become more prevalent, as it posesses major theoretical and practical advantages over rival methods such as PCA and correlation analysis (Gauch and Whittaker, 1981; Ter Braak, 1986; Dixit *et al*, 1989; Varis *et al*, 1989). This study was not initiated with the application of CANOCO in mind as it became available only in 1988, and therefore there are some deficiencies in the data used with CANOCO. Future studies should be carried out considering the requirements of CANOCO. In particular it must be considered that a canonical analysis can be carried out only using a number of environmental variables which is two less than the number of sites. Therefore as large a number of sites as possible should be sampled. Also the quality of the environmental data is important. CANOCO cannot analyse data sets with missing values, so it is necessary to ensure that all variables are measured at all sites, otherwise estimates must be inserted which inevitably reduce the quality of the analysis.

The measurements of PAR carried out in this study were of necessity limited to a few isolated sites, which restricts the value of this variable in the CANOCO analysis. Since PAR is a variable of major importance in the growth of algae it would be preferable to measure it at all sites throughout a study. This would require the use of a large number of instruments which, considering also the public access to some sites, would need to be cheaper than those developed for this study. The instrument described by Newman (1985) would appear to satisfy these criteria.

Mean site temperature cannot be adequately measured by spot-sampling with a thermometer, as revealed in the results (Fig. 25b). A better means of recording mean temperature is thus necessary, using either data loggers or the instrument described by Woodward and Yaqub (1979).

If the community composition is truly a reflection of the environmental regime, then changes with time, for example of mean pH in the L. Ard streams, ought to result in predictable changes in the relative abundance of algal species. Sampling these sites at some later time, using the same techniques, would reveal whether this was the case, by permitting pH values inferred from the species data to be compared with pH measured contemporaneously.

It is also important that the conclusions reached on the basis of the Loch Ard sites should be checked by using comparable species data from streams in other areas, with a wider range of mean pH values, or in areas with different geological, topographical and rainfall characteristics.

Finally, if the findings of laboratory studies on the autecology of algal species are to be successfully used in interpreting the species values derived from the application of statistical procedures such as CANOCO to natural populations, measurements of growth rate should be carried out *in situ* in natural streams, with measurement of the relevant environmental variables carried out simultaneously and preferably continuously. The techniques for measuring growth rates described here could be applied also in a situation where a field laboratory can be employed.

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APPENDIX 1

SPECIES DESCRIPTIONS

Taxon no. Cyanobacteria: Oscillatoriaceae

1   *'Oscillatoria '* 1 µm diameter

Little detail of this species is discernable in field samples, but it may be *Pseudanabaena* sp. which has been found to grow in cultures, as a frequently significant contaminant of other algae. This has been found to grow in experimental conditions of low pH (4.0) and high [Al] (200 $\mu g l^{-1}$ at pH 5.5). Motility has not be observed. In field samples this 'taxon' probably includes several species.

2-6   *Oscillatoria* species

These are cyanophytes growing as long straight filaments, showing no branching or heteocyst formation. They may occur as dense mats, like *Phormidium* spp. but more frequently are found intermingled with other algae, more dispersed than *Phormidium* as their distinguishing feature is the possession of motility.

This however is not always exhibited during examination so that a degree of uncertainty may exist between *Oscillatoria* and *Phormidium* in certain cases.

West (1904) concluded that the genus *Phormidium* may serve as a receptacle for species which cannot be strictly referred to *Lyngbya* and *Oscillatoria.*

Varieties encountered have been somewhat arbitrarily divided into size groups as 2, 5, 8, 11 and 14 µm. diameter. Variations in colour are also found but these have not been taken into account.

7-10   *Phormidium* species

These filamentous cyanophytes are non-motile and possess a thin sheath (not always visible). They are usually found in almost unialgal dense mats, sometimes with a mixture of other species, especially *Oscillatoria.*

They have been divided into the size groups 3, 4-5, 8 and 13 µm diameter, taking no account of colour, as this can vary within a mat due to the effects of self-shading.

11   *Tolypothrix*

This is a filamentous cyanophyte, approximately 11 µm. diameter within a dense sheath which becomes dark brown with age.

The alga exhibits false branching, with a heterocyst always being found at the point of branching. Hormogonia may be formed composed of a small number of cells separated from others within the sheath. Isolated portions of the filament may therefore be readily confused with species of *Lyngbya* , if the false branching is not seen. The cells may be turquoise in young filaments but tend to turn brown with age.

12 In its most reliable location (Burn 6) *Tolypothrix* frequently possesses an epiphyte, a greyish cyanophyte filament approximately 3 µm diameter and greyish in colour, with no sheath or cell differentiation visible. This may be *Chamaesiphon* sp.

13 *Stigonema*

This cyanophyte is composed of cells closely appressed into a multiseriate filament, with small branches emerging from it initiually as small knobs or swellings. The colour is dark green, frequently appearing black. It is more frequently found in damp, aerial locations than actually under water. It may form dense 'mossy' expanses beside waterfalls, etc.

14 *Scytonema*

This is a sheathed filamentous cyanophyte similar to *Tolypothrix* but having a different form of false branching, two false branches emerging from the sheath at the same point. Again if this feature is not seen the sample may be ascribed to the wrong genus.

Rhodophyta

15 *Batrachospermum*

This is a large alga. It may be readily seen with the naked eye and its form is visible with a hand lens or low power microscopy. The colour may be dark brown or deep turquoise The main axis of the plant is composed of many layers of cells with whorls of branches emerging at intervals giving a beaded appearance. The plant is invested in mucilage, making the gathering of samples quite difficult. It has not proved possible to grow this alga in the simple inorganic media used in this study.

16 *Lemanea*

Another Rhodophyte, this is also large enough to be readily seen with the naked eye. It grows firmly attached to rocky substrata in locations exposed to permanent rapid currents. The thallus is multiseriate, tough and wiry, greyish in colour, tapering towards the tip with swellings (nodes) at intervals.

17, 18 *Audouinella* (*Rhodochorton*)

This Rhodophyte frequently forms a short pinkish turf on stones in fairly fast-flowing water. The filaments are branches, blunt-ended, with cells approximately 17 µm in diameter and a pinkish coloured chloroplast distributed around the periphery of the cell. Less frequently a variation is found in which the chloroplast is greyish or grey-green. This may not be a separate species.

Chlorophyceae: Chaetophorales

19 *Draparnaldia*

This chaetophoracean has a main axis of cell approximately 50 µm. diameter with branches arising at intervals composed of narrower cells. These branches are subdivided to give a tufted appearance. The terminal cells are usually drawn out into a long colourless hair. The chloroplast is a parietal band, fimbriate in the large cells of the main axis.

20, 21 *Stigeoclonium*

This chaetophoracean resembles *Draparnaldia* in the shape of the chloroplast and the possession of terminal hairs but shows no main axis differentiation and branching tends to be simple. There are numerous problems in the identification of *Stigeoclonium* species but for operational purposes two types have been recognized. The first has filaments composed of cells approximately 5-7-(8) µm in diameter, while the second has cells 8-14 µm in diameter.

Oedogoniales

22-24 *Oedogonium* species

The cells are slightly tapered so that the entire, unbranched, filament becomes wider with distance from the holdfast. Bulbous, oogonial cells may also be found and the cell walls bear ring-like scars due to the method of cell division. In some cases the narrowest filaments (approximately 6 µm diameter) may show little tapering and confusion is possible with other genera. Three size classes have been distinguished; 6 µm, 15 µm and 30 µm. There is the possibility of overlap between the 15 µm class and the other two due to the tapering nature of the filaments so these are purely operational taxa.

In culture these taxa have been observed to produce zoospores, settle on substrata and grow for several days but have not continued to grow indefinitely.

Zygnemataceae: Zygnemales

25, 26 *Spirogyra* species

These have been found entangled with other vegetation rather than actually attached to the substratum and have not been observed in culture. The cells are cylindrical, the filaments long, uniform in diameter and with clearly visible spiral band chloroplasts. Two size classes were found in L. Ard samples; diameters approximately 20 and 32 µm. They are not very common in these samples.

27, 28 *Zygnema* species

These are usually distinctive, being broad (approximately 20 µm or 28 µm diameter) filaments, with twin stellate chloroplasts, dark green under the microscope but the mass of filaments appearing almost brown to the naked eye.

Cell diameter is reported to be an unreliable taxonomic feature in this genus (Miller and Hoshaw, 1974).

A morphological variant was rarely encountered in L. Ard samples, and from other areas, in which the chloroplast is a parietal ribbon or band, the full length of the cell, but filling approximately 50% of the circumference so that it appeared similar to *Mougeotia* or *Ulothrix* spp. That it was actually *Zygnema* was revealed when a filament was discovered with normal *Zygnema* morphology at one end but the aberrant form at the other. There were no pyrenoids visible in the aberrant chloroplast.

29-33 *Mougeotia* species

Mougeotia species have an axial band chloroplast with several pyrenoids. The criterion for species identification rests on the morphology of the zygospore so that it is not possible to determine the species of most field specimens. The filaments are essentially unbranched but occasional short branches can be found in taxa 30-33, originating at a Y-shaped cell. The lateral outgrowth may have a somewhat irregular appearance and sometimes appears to be functioning as an additional holdfast cell. In culture *Mougeotia* species were not observed to develop from spores but from hormogonia. One end of the filament appeared to develop the ability to attach to the substratum, and in the case of the narrowest cell type, (5-)8 µm diameter, the basal cell was almost always found to form a recurved hook-like structure. Larger taxa attach by means of a cell with irregular outline as described above.

The operational taxa are:

29 = 5-8 µm. possibly *M. viridis* (Kütz.) Wittr. (may include other species also)

30 = 11µm

31 = 14-17 µm. possibly *M. capucina* (Bory) Agardh.

32 = 20-22 µm.

33 = 25-28 µm.

Taxon 29 has a chloroplast with a very clear, non-granular appearance, unlike the others. Taxon 31 frequently has cytoplasm tinged purple, indicating *M. capucina.* The identifications are after Randhawa (1959).

(Chaetophorales)

34 *Microthamnion*

This is a microscopic branched filament, with cells approximately 3 µm. in diameter, branching frequently but with short branches, the terminal cells blunt with a single parietal chloroplast occupying approximately half the cell's circumference. The whole plant has not been found, in this study, to be more than 1 mm. in length. Its taxonomic relationships are uncertain but it is classified as a Chaetophoracean by Prescott (1970).

Microsporales

35-38 *Microspora*

Microspora species are unbranched filaments, some at least having the capability in culture to attach to surfaces by a specialized basal cell, following zoospore germination. In field samples however they are almost always found entangled in mosses and liverworts and their original point of attachment is not discernible. Their distribution is therefore practically limited to those waters where mosses and liverworts are plentiful.

The cells are encased in walls composed of H-shaped segments, and in the species found, these segments may normally be distinguished under the microscope. However senescent plants show a degenerate morphology in which the chloroplast, normally a plate or complex network filling the cell, becomes contracted and the wall material becomes swollen and hyaline. The H-segments may be invisible, making the morphology of the filament similar to that described for *Binuclearia*, as discussed in section 4. As *Geminella* spp. may also undergo degenerative changes to somewhat resemble *Binuclearia* there is a possibility of confusing *Microspora* and *Geminella* species. This is primarily a problem with the 8 µm wide taxa of these two genera. The taxa of Microspora recognized in this study are:

taxon 35 - *Microspora* 8 µm. diameter; possibly *M. tumidula.* Hazen
36 - *Microspora* 1 µm. diameter
37 - *Microspora* 14 µm. diameter
38 - *M.icrospora* 22 µm. diameter.

Ulotrichales

39,40 *Geminella* species

This genus is distinguished by the possession of a mucilage sheath, described as wide (Prescott, 1970), but in the two examples found in the Loch Ard area the sheath is relatively narrow.

Apart from the possession of this sheath the filaments resemble *Ulothrix* species, but the cell walls are very indistinct so that the appearance is of Ulotrichoid chloroplasts strung together in a clear refractile tube.

Some species of *Ulothrix* are reported to possess narrow sheaths (Ramanathan, 1962) but none of these appear to resemble the species described here.

In nutrient depleted conditions the cells may become widely separated within a clear matrix. In this form they resemble algae of the genus *Binuclearia.* The two taxa distinguished have cell diameters (6)-8 μm. and 10-11μ m. With these dimensions both would belong to the species *B. tectorum* Kützing (Ramanathan, 1962).

However as this morphology is only found in senscent samples (e.g. old cultures or field samples gathered in summer) I am reluctant to ascribe any samples to the genus *Binuclearia* and have adopted the working designations *Geminella* 8 μm. and *G.* 11μ m. They may be size variants of the same species.

41, 42 *Hormidium* species

Two taxa are recognized, modal cell diameters approximately 5.5 μm. and 8 μm. They have long filaments of cylindrical cells, not usually constricted at cross-walls. Taxon 41 ranges from 5 to 7 μm. diameter and taxon 42 ranges from 7 to 9 μm., so there is a degree of overlap and if only one is present in a sample it may not be possible to unequivocally ascribe it to the 'correct' taxon. These are operational taxa and may both belong to the same species.

The cells are 1-2 times as long as broad, occasionally longer. The cell wall is thin, with no sheath. The chloroplast is a parietal disc, covering about half the circumference and 50-100% of the cell length. Frequently two chloroplasts per cell are found, especially in the longer cells (longer than 2 cell diameters).

In a nutrient depleted culture this form of the chloroplast is easily seen, but in a fresh culture or in nutrient sufficient conditions in field samples the chloroplast is enlarged and may appear more Ulotrichoid. In nutrient depleted conditions the cytoplasm is also seen to be more granular in appearance and the cells tend to be longer.

43-49 *Ulothrix* species

These taxa have cylindrical cells forming long unbranched filaments. The chloroplast is a parietal ring, which is incomplete in the smaller taxa, covering about half the circumference and the full length of the cell. There is thus a great deal of overlap possible with the *Hormidium* species described previously.

Taxa ascribed to the genus *Ulothrix* have the following descriptions and dimensions:

43 8 µm. diameter, may not be a separate species from 42, and is distinguished only by appearing 'more Ulotrichoid'.

44 10 µm. diameter. Similar in appearance to the last taxon, but larger. The chloroplast occupies the full length of the cell.

45 10-11 µm. diameter, with walls frequently bearing plates of thickened material, often coloured deep brown so that the cell contents are obscured. The form of the chloroplast is similar to the previous taxon. This is believed to be *Hormidium crenulatum* Kutzing, as described by Ramanathan (1962). The operational designation '*Ulothrix* 10 µm. scaly' has been retained in figures and tables.

46 14 µm. diameter, chloroplast a complete ring.

47 17 µm. diameter, chloroplast a complete ring.

48 25-30 µm. diameter, chloroplast a complete ring.

49 36 µm. diameter, chloroplast a complete ring.

The last four taxa are uncommon in the L. Ard samples.

APPENDIX 2

LIGHT MEASUREMENT

The electronic circuits desscribed in this appendix were developed and built by John Gurney, of Edinburgh. Full circuit diagrams of the integrators are therefore not provided here, being his intellectual property, but the direct-reading meter circuit is shown with his permission.

(a) Sensor design
(b) Instantaneous PAR measurement
(c) Integrator design
(d) Integrator construction
(e) Sensor calibration
(f) Integrator calibration.

(a) Sensor design

In measuring Photosynthetically Available Radiation (PAR) it must be borne in mind that this is only a fraction of the available light energy supplied in sunlight, since plants respond to light between 300 and 700 nm. with a peak in the blue and a peak in the red region. It is therefore not appropriate to measure total energy available, for example with a solarimeter, and even comparisons between two sites may not be possible with such an instrument if the light quality is different, for example because of a forest canopy.

To accurately measure PAR a sensor should have the same sensitivity response as the action spectrum of photosynthesis in the plants it is desired to study. Furthermore measurement should be in units of photons rather than energy, since photosynthetic reactions start by the capture of photons, which elicit the same response no matter the energy which they contain.

Barrier-layer semiconductor devices can be used as sensors. Silicon photocells have been widely used as they provide an output which is proportional to the number of quanta of incident light. However they require to have a large surface area, and are therefore expensive, in order to provide enough electrical output to be measureable.

An alternative is the photodiode, which will provide a small amount of power in response to light, but which can also be reverse-biased and will then provide an output current proportional to incident light (expressed as quanta) which can be amplified.

The spectral response of photodiodes and photocells does not match the action spectrum of plants, and filters must be used to correct this. Commercially available sensors are designed to two different response shapes. A quantum sensor is designed to give a flat response between 300 and 700 nm and to have a sharp cut off below 300 and above 700. A PAR sensor is designed to show a biomodal response with a peak at 400 nm and another about 620 nm, the actual shape of which is a match for the mean of action spectra for photosynthesis in 22 land plants (McCree, 1973).

Such a sensor may not therefore accurately mirror the response of an individual species, and there may be an even greater discrepancy with aquatic plants and single-celled or filamentous forms. It was therefore decided to base the design response around some of those species of fresh-water algae being studied. Since filamentous green algae were the main focus of the experimental work, these were chosen as the models for sensor response. The ideal basis for designing a sensor is the photosynthetic action spectrum, but in cases where this is not available, for practical purposes the absorbance spectrum of a cell suspension can be substituted (Kirk, 1983).

The absorbance spectrum of *Geminella* 8 µm. was measured in a Unicam SP8001 double-beam recording spectrophotometer according to the recommendations of Kirk (1983). The filaments were sonicated to yield a cell suspension and measured with a diffuser between the suspension and the photocell to correct for light scattering, against a blank with a similar diffuser. The diffusers were dense white acrylic which showed very little differential absorbance with wavelength. Use of two diffusers in the double-beam machine ensured that this would cancel out in any case.

The spectra of suspensions of other species were measured at a later date. The *Geminella* results were used for the sensor design. The absorbance curve of the algae was found to be close to that of chlorophyll extracts, with a pronounced dip between the two absorbance peaks, confirming the need to design a better sensor response than one based on McCree (1973) (Fig. 71).

The basis for the sensor is the BPW21 photodiode (Radio Spares) which is designed to have a spectral response similar to the human eye and incorporates a Schott glass filter. The response was modified by the use of a Kodak Wratten CC20M and a Cinemoid 71A acetate filter. These were chosen on the basis of the calculated product of the proportional transmission at 10-20 nm intervals and the proportional response of the photodiode. Matches between the resultant sensitivity profile and the design criterion were checked by trial and error using transmission data for different filters.

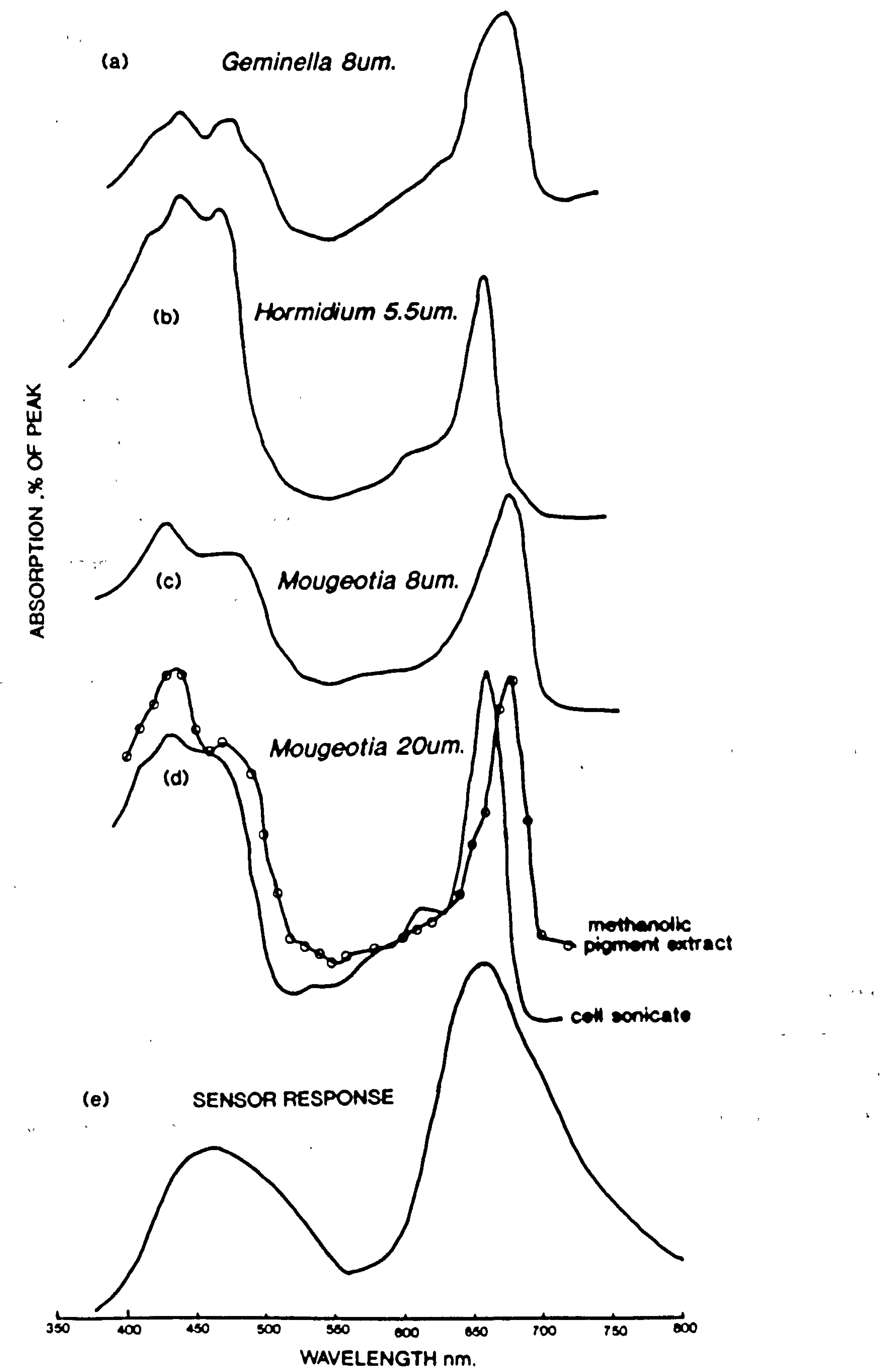
The response profile of the final sensor was tested by Macam Photometrics Ltd., Livingston, and found to match the calculated pattern (Fig. 71c). This is not an exact match for the *Geminella* absorbance spectrum due to a miscalculation of filter transmittance made early in the design process, but is reasonably close and in any case must also serve to measure light reaching other species. It is likely that the match would be less good for diatoms, Rhodophytes and blue-green algae.

The diode and filters are mounted beneath a white acrylic diffuser (dense white Perspex) in a sensor head based on the design of Biggs *et al* (1971). This is designed to cosine-correct the response of the photosensor; at high angles of incidence little light is absorbed via the side of the diffuser disc, but as the angle decreases this proportion increases. When the light becomes horizontal to the plane of the sensor head, the rim of the head cuts off the beam completely. The design was modified slightly by angling the annular trough around the sensor and incorporating drain holes for field use, so that rainwater would not accumulate and cause inaccuracy by reflection.

The heads were cast in epoxy resin with black pigment and an inert filler. Calcium carbonate or iron powder were used as fillers. The iron has the advantage that it renders the casting conductive and thus it can be connected to the screen around the photodiode lead, improving the screening against R.F. interference. This is considered a potential problem in the field because of thunderstorms and particularly because some sites lie beneath high voltage power lines which can discharge to earth in some atmospheric conditions.

Slight shrinkage of the casting occurs during setting, and the hardened castings were hand-dressed back to the design dimensions. The Perspex diffuser discs were glued in position and the top surface levelled with the rim of the head. The filters were mounted dry between the diffuser and the photodiode, and the diode anchored in position with epoxy cement. The diode leads were then soldered on and the central cavity sealed with silicone rubber. Finally a base section of epoxy resin of the same composition as the head was cast over the inverted head. The completed heads were glued to a small acrylic plate which could be bolted to the Dexion tile racks in the field.

FIG. 71: QUANTUM-CORRECTED ABSORPTION PROFILES OF CELL SUSPENSIONS USED IN DESIGNING SENSOR RESPONSE CURVE.



(b) Instantaneous measure of PAR

The sensor head can be used with a modified voltmeter to measure instantaneous incidence of PAR. The calibration was carried out at 660 nm by Macam Photometrics Ltd, in energy units and a correction applied to convert to quantum units.

Comparison of readings taken with this instrument in daylight and simultaneous readings with a Skye PAR meter (SKP200) show that the Skye instrument gives approximately 25% higher values (μmoles $m.^{-2}s.^{-1}$) in daylight, 60% higher in fluorescent light, but similar values in tungsten light.

The circuitry used for direct reading is shown in Fig. 72.

(c) Integrator Design

The integration of measured Photosynthetically Available Radiation is desirable in ecological surveys for the comparison of field sites which may be subjected to different degrees of insolation at different times of the day. PAR may be detected using a photodiode and suitable filters. Integration may be achieved in three different ways:

(i) by sampling intensity at frequent intervals and storing the information in a data logger
(ii) by electronic integration, resulting in a 'count'
(iii) by chemical integration.

Use of data loggers may be limited by their high cost, especially on sites which may be susceptible to accidental damage or interference. Chemical integration (Newman, 1985) may be the cheapest option if a large number of sites are to be monitored. For a modest number of sites, independent electronic integrators may be the best option. We describe two circuits which can be built for less than £65 each (1988 prices), which are weatherpoof, unaffected by temperature, will integrate linearly down to 0.1% of normal daylight levels, and may be left unattended for months at a time.

Two different electronic integrator circuits were built. The first is a modification of the instrument reported by McLaughlin and Allan (1976). Their instrument uses a reverse-biased photodiode which supplies a current dependent on the light intensity to a capacitor. The capacitor is charged up to a pre-set voltage at which point the capacitor is grounded by a relay and a pulse sent to an electromechanical counter.

They employed a range switch to bring in different value capacitors in order to cover a range of light intensities. This would cause problems in an instrument left unattended for long periods as a low-value capacitor would lead to count rates which were in excess of the performance of the counter while the large-value electrolytic capacitors they employed for the high light intensities would give inaccurate counts at low light intensity because of the high leakage rate inherent to these capacitors. Our modification minimises these problems by the use of polycarbonate capacitors which have a very low leakage rate.

Another protential problem arises at high count rates where a capacitor may not be fully discharged in the short time taken by the relay to change from one state to the other, thus leading to an overestimate of PAR. Also, under these conditions, loss of charge may occur due to the diode being disconnected from the capacitor during switching ('dead' time). We have minimised these problems by employing two capacitors which are charged and discharged alternately. The diode is thus only disconnected very briefly and we believe that minimal loss of charge occurs. Each capacitor has as long to discharge as to charge. Capacitor values and trigger point potential were chosen to give, in conjunction with the sensor characteristics, a count rate at maximum natural light intensity which was well within the switching capacity of the electromechanical counter.

The device is shown in block diagram form in Fig. 73. Two latching relays switch input and output to the two integrating capacitors alternately. The capacitor voltage is fed to a voltage follower which has an input impedance in excess of 10^{10} ohms. Current leakage of the stored charge is thus negligible. The output of this voltage follower is fed to a comparator with a trigger point settable by a potential divider at around 4.5 v. When the trigger point is reached, a pulse is sent to the bistable latch. The two outputs of the bistable go high alternately with successive pulses. They are connected to monostables M_1 and M_2 which produce a discrete pulse each driving a transistor and feeding the integrating current to one side or the other of the integrating capacitor relays. One of these relay lines is also connected to the electromechanical counter which thus registers a count each time a full cycle is completed.

The op-amps (voltage follower, latches and comparator) are supplied with a regulated 6 v from a 15 v battery pack. The voltage regulator incorporates temperature compensation so that the trigger point is not influenced by ambient temperature, making burial in the ground (e.g. Woodward and Yaqub, 1979) unnecessary. Tests performed at -18°C to approximately 40°C revealed no detectable effect on count rate, although the instrument was not exposed to the lowest temperatures for sufficient time for the battery pack to reach this temperature.

The relays and counter are powered directly from the battery pack. Their operating range is from approximately 10 v to 15 v so that the cells can be run down to 1.0 v or less each before the instrument will fail. At this point the op.amps are still being supplied with a regulated 6 v. Another consequence of powering the relays and counter directly is that no power surges occur in the regulated line. A large decoupling capacitor is incorporated in the 15 v line.

Use of CMOS circuitry has reduced the power consumption to 90-1,000 μA depending on count rate. A battery pack of Duracell AA cells has operated for approximately 1 year in field use.

The second design of integrator (Fig. 74) was initially built in order to check the behaviour of the photodiodes in reverse-bias mode as opposed to open circuit, as the manufacturers data refers to open circuit current being linear with light intensity. The design also incorporates temperature compensation of the trigger point by floating zero and bears some similarity to the instrument described by Saffell *et al* (1979).

The sensor is connected via a polarity reverse solid state switch to a current-voltage converter I.C. The diode is thus in open circuit mode. The voltage output is proportional to the current input. This voltage is fed in turn to an integrator op-amp. The integrator produces a rising or falling voltage ramp, according to the polarity of the voltage input. The height of the ramp is controlled by a Schmitt trigger set for ±3.6 v. Thus when the ramp reaches + 3.6 v the Schmitt trigger changes state. This causes the polarity sensor to drive a bistable from one state to the other which in turn reverses the polarity of the photodiode and thus reverses the direction of the ramp. The voltage then falls to -3.6 v, when the polarity is reversed once again and the cycle is completed.

It was found that the pulse rate had to be divided by 10 since the calculator used to accumulate the count was unable to operate at frequencies in excess of 1 Hz. The divider output is fed to CMOS analogue switches which send alternate signals to the '1' and '+' switches (buttons) of a cheap digital calculator.

It is necessary to include additional circuitry with battery-operated calculators to prevent the auto-off facility turning the display off and thus losing the count at low light levels, e.g. at night. An alternative is to use a solar-powered calculator, and connect it to a single dry cell instead of the solar power pack. The integrator operates on a regulated ± 6 v supplied from two 9 v packs.

This integrator operates at a higher frequency than the first design. P.D. measured at the Schmitt trigger shows a symmetrical saw-tooth profile above and below the zero line. Temperature effects on supply voltage will cause the zero line to shift but the waveform will be unaffected since both the + 6 v and the -6 v supplies will be shifted in the same direction by the same amount.

(d) Instrument construction

Exposure to adverse climatic conditions was taken into account in constructing the integrators. Burial in the ground was adopted by Woodward and Yaqub (1979) as a protection against temperature extremes and would probably reduce the likelihood of some other types of damage but was considered impractical in many of the sites at Loch Ard. Therefore the instrument had to be insensitive to temperatures from below zero to possibly over 40°C, for as shown in preliminary tests these temperatures could be achieved in strong sunlight with an unshaded integrator box. It was found that some mercury-wetted relays used with the integrators would not operate at elevated temperatures, and these had to be changed. As a further precaution, metal shades were constructed to cover the instruments, which gave protection against rain also. The circuitry was enclosed in R.F. screened ABS boxes (Radio Spares) since it was anticipated that interference could occur due to electrical storms or proximity to pylon lines. Switches, counter displays and battery test jack sockets were mounted through the box and thus further protection was required. The ABS box was enclosed in a polyethylene sandwich box and screwed to a wooden board along with the solar shade. The board could then be mounted vertically on Dexion stakes. The ABS box was earthed through the stakes and the earth screen on the diode lead also grounded.

The boxes could not be hermetically sealed, since the diurnal temperature fluctuations would lead to a 50% expansion in the volume of air contained, which could lead to the collapse of the boxes, or the possible entry of water through a leaking diode cable, during cold weather. Instead the boxes were deliberately vented through the jack sockets used for battery testing, via a vial of silica gel which could be replaced each time the instrument was visited. A second vial was sealed inside the box. In practice it was found that leakage of air did occur and the vial inside also required to be changed occasionally.

If necessary in very adverse conditions the mechanical counters can be read through the lid of the sandwich box.

(e) Calibration of sensors

One sensor was calibrated by Macam Photometrics Ltd, at a sensitivity of 1.67×10^{-7} A.W.$^{-1}$ m.$^{-2}$, at the peak, 660 nm. This is equivalent to 0.0303 A. µmole $^{-1}$m^{-2}s^{-1}, which is thus the sensitivity, in quantum terms, across the whole spectrum.

This sensor is employed with the direct-reading meter for instantaneous PAR measurement. The meter reading is in millivolts, and calculation of the sensor output takes into account the value of the range resistor employed (27K, 270K or 2.7 M Ω).

The relationship between mV reading and PAR is thus:

$$\text{PAR, } \mu\text{mole m}^{-2}\text{s}^{-1} = \frac{\text{mV} \times 103}{2.7 \times 10^{6} \times 0.0303}$$

$= \text{mV} \times 0.0122$ (for the 2.7 M Ωresistor, the most sensitive range).

Comparison of PAR measured with this instrument and a commercially available PAR meter (Skye SKP200 with SKP210 sensor) shows that the home-made sensor gives a lower reading in sunlight and fluorescent light, but almost identifical values in tungsten light.

The sensor response (Fig. 71e) was intended to match the absorption profile of the green alga *Geminella* 8 µm., but due to miscalculation the fit is not as good as expected. It is also apparent from Fig. 71 that a sensor which fits one species may not properly estimate the PAR available to another even within the Chlorophyta. Due to the simple structure of these algal cells, the absorption profiles of algal suspensions are very similar to those of pigment extracts (Fig. 71d) but more complex forms found in the Rhodophyta for example may in addition to other pigments have the response modified by the multiseriate structure (*Lemanea*) or the presence

(f) Calibration of PAR. integrator

Checking linearity: method

Linearity was checked against a pair of Kipp Solarimeters simultaneously subjected to an equivalent light intensity. Intensity at the location of each sensor head was checked, in the case of artificial light sources, by comparing with an instantaneous reading PAR meter.

A series of readings was taken for each light source at different intensities (obtained by altering the distance from the light source). It cannot be assumed that different sources will have the same spectral distribution, so that the ratio between Kipp count and PAR count may differ. Light sources used were:

Tungsten light	6 x 100 watt bulbs
	4 x 100 watt
	2 x 100 watt
	2 x 60 watt
	2 x 40 watt
Natural light	Full sunlight
	Full sunlight enhanced by reflection.

In practice it was found that all combinations of 100 w bulbs gave the same ratio, and 60w bulbs gave the same as 40 w. A high, even intensity of light was only obtainable over the entire area of the sensor array using direct full sunlight. This was increased by about 50% using reflection from two sheets of aluminium foil. The reverse (least shiny) side of the foil gave a reflection free of high spots.

Linearity of response was checked by calculating the correlation (i) between Kipp count rate and PAR count rate and (ii) between the ratio and Kipp count rate (Table 56a).

(i) gives a figure of between 0.992 and 1.000

(ii) gives a low or negative correlation, showing that there is no tendency for the measured energy - PAR relationship to vary with light intensity, i.e. no consistent non-linearity.

The different values of 'b' in the regression equation found with different light sources (Table 56b) is a reflection of the different spectral composition of the light. The Kipp solarimeters respond equally to the energy content of photons throughout the wavelength range whereas the PAR sensor has the complex pattern shown in Fig. 71e (shown in quantum terms).

TABLE 56

LINEARITY CHECKS ON PAR INTEGRATOR

(a) CORRELATIONS BETWEEN KIPP AND PAR INTEGRATOR COUNTS

Source	(i) Correlation Kipp/PAR	(ii) Correlation Ratio/Kipp
100 w	0.999	-0.051
60 w + 40 w	1.000	-0.612
Combined Data Tungsten light	0.999	
Sunlight	0.999	-0.408
Enhanced sunlight	0.992	0.023
Total sunlight data	1.000	

(b) REGRESSION OF PAR ON KIPP COUNT

MODEL: PAR count =		b (Kipp count)	= a + b (Kipp count)
100 w	=	0.0932 Kipp	= 0.0060 + 0.0928
60 + 40 w	=	0.0845 Kipp	= 0.00228 + 0.0854
Enhanced sunlight	=	0.300	= 0.090 + 0.299
Sunlight	=	0.302	= -0.293 + 0.308
Total sunlight	=	0.300	= 0.124 + 0.298

The lack of consistency in the 'a' term found in the second form of the regression suggests that there is little or no dark current effect or loss of stored charge from the integrator capacitors (leakage), nor any similar flaw in the Delta-T devices integrator (Type MV1) used with the Kipps.

This testing was carried out on the twin-capacitor instrument. Linearity checks were also made on the ramp integrator, and all integrators were checked together prior to field deployment by exposing them on the Polytechnic roof with all sensors set at the same level, so that no shadows could interfere with the light reaching any instrument. The results of these tests were also used to calculate a factor to relate the counts obtained on the different instruments. (The rate is adjustable by varying the preset voltage value at the Schmitt trigger, but cannot be perfectly matched between instruments over a long period).

Some variability in the value of this factor was found as the calibration was carried out in winter, with low angles of incidence and low intensity of natural light. This indicates some variation in the cosine correction provided by the different sensor heads, due to slight differences in manufacturing tolerance. There was less variability when all heads were tilted towards the south, and in practice throughout the year the error due to this flaw would be quite small, although it has not been estimated.

Calculation of a factor relating the integrator count of the reference instrument to an absolute value of PAR was carried out by comparing the integrated count with an instantaneous reading under artificial light.

FIG.72: CIRCUIT FOR DIRECT-READING PAR METER

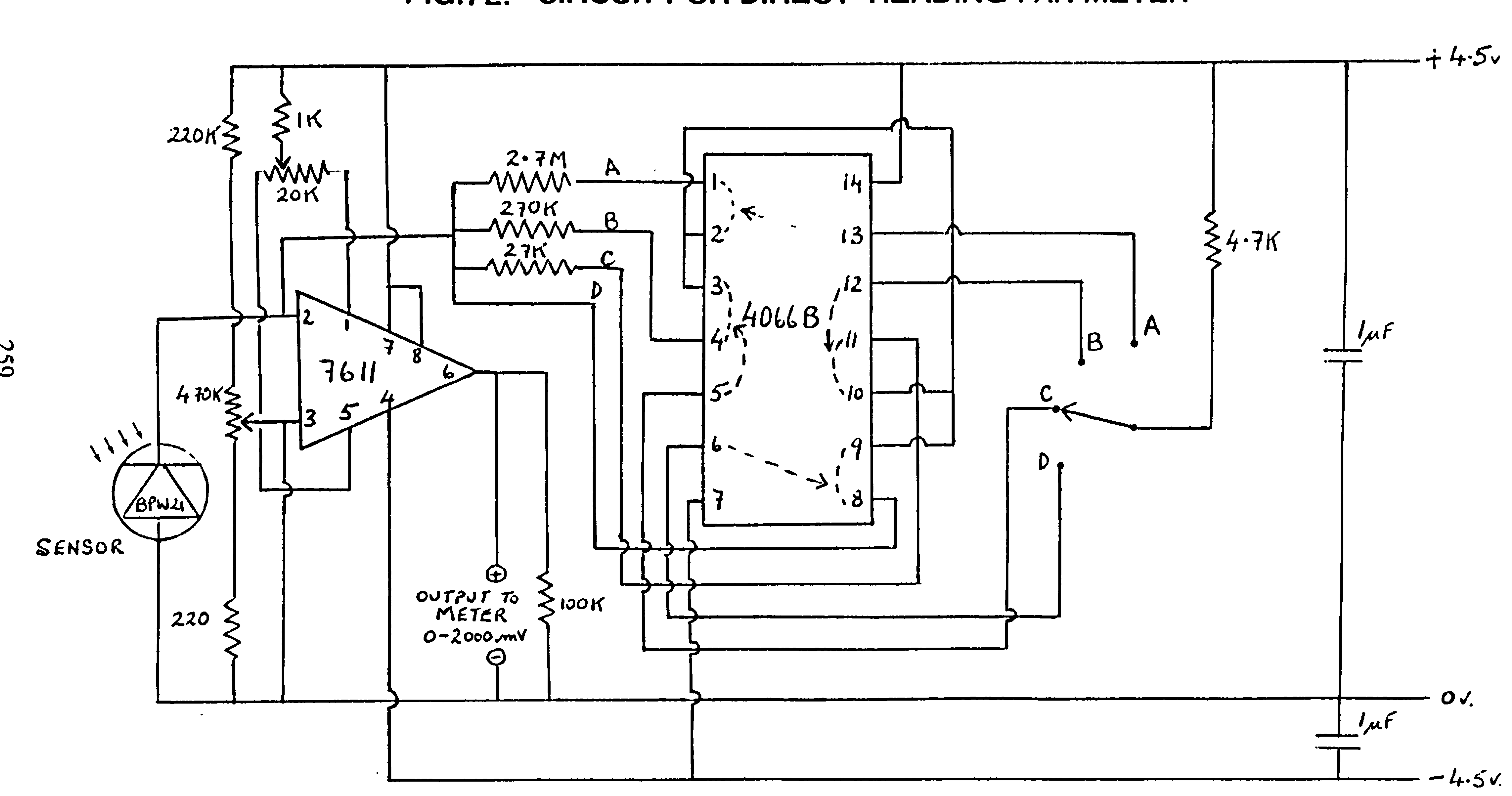


FIG.73: TWIN CAPACITOR CURRENT INTEGRATOR

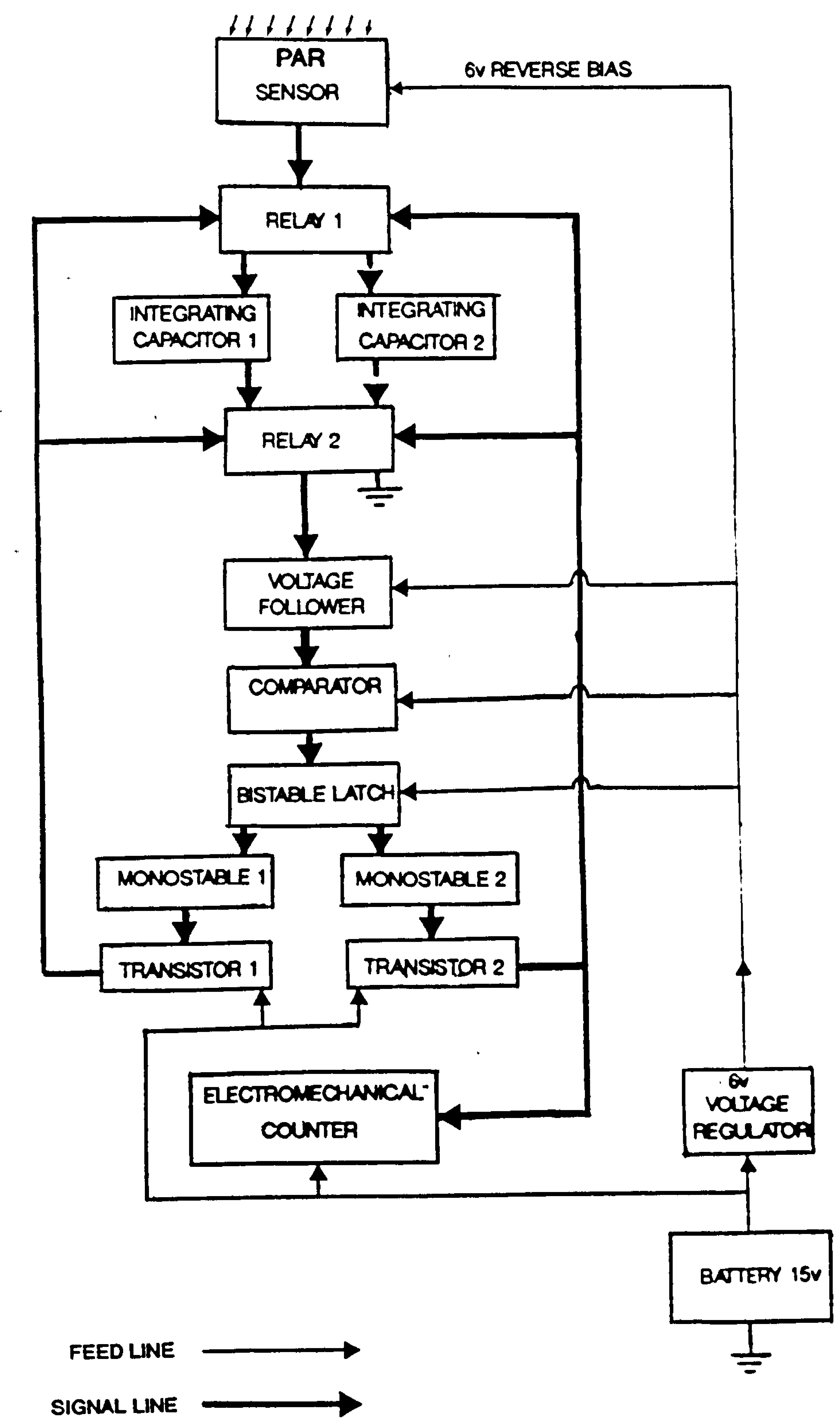
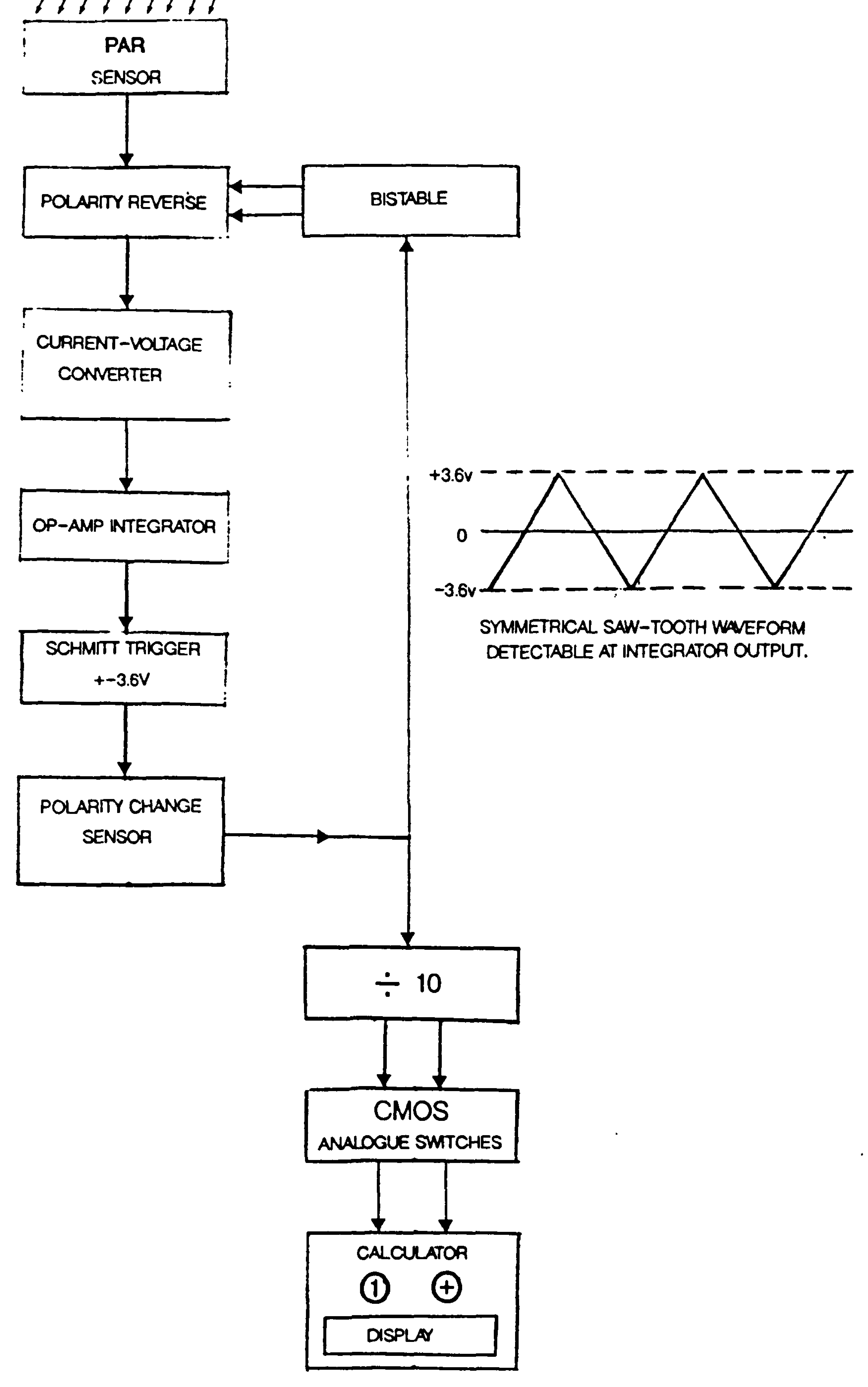


FIG.74: VOLTAGE RAMP CURRENT INTEGRATOR



APPENDIX 3

FIG.75: CIRCUIT FOR THERMOSTAT USED IN CHANNEL APPARATUS

9v CHANGEOVER RELAY
LINE OUT TO REFRIGERATOR
LINE OUT TO HEATER (ALTERNATIVE USE)
200Ω COIL
L
N
E
240v A.C.
12VA
+9v
0v
+9v
IN400Z
IN400Z
+12v NOMINAL
470Ω
20v,400mW ZENER
2200μF
IN4148
IN4148
BC109C
VOLTAGE REGULATOR
220nF
220nF
100μF
+8v REGULATED
0v
3.3K
470K
3130
10K
B
10K
A
BAT85
BAT85
1K
BEAD THERMISTOR
NTC 10K @ 25°C
1K FINE ADJUST
10K COARSE ADJUST

POWER SUPPLY, +8v.

VOLTAGE REGULATOR - 78L08

ADJUSTABLE HYSTERESIS:

A - SETS UPPER TRIGGER POINT 6.5v
B - SETS LOWER TRIGGER POINT 6.35v