ACCUMULATION OF HEAVY METALS BY AQUATIC BRYOPHYTES IN STREAMS AND RIVERS IN NORTHERN ENGLAND

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This thesis results entirely from my own work and has not previously been offered in candidature for any other degree or diploma.

John D. Wehr

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to Deb and Sarah

ABSTRACT

A study was made of the ecology of aquatic bryophytes and their accumulation of metals in rivers of northern England. Field surveys and experiments in the field and laboratory examined the effectiveness of bryophytes as monitors of heavy metal pollution.

A survey of 105 river sites (10-m reaches) with Rhynchostegium riparioides was carried out, together with a seasonal survey of this species at seven sites, which also included data for two other species. Details of the aquatic bryophytes present, water chemistry and metal concentrations in mosses are given. The ecological ubiquity of Rhynchostegium was described using principal components analysis and discussed in relation to other macrophytes. A biometric study revealed that marked interpopulation differences in gametophytic characters were correlated with water chemistry variables (NH_4-N, PO_4-P, Cl, Na) indicative of organic pollution.

Significant linear regressions were found between accumulation and aqueous concentrations of Zn, Cd, Ba and Pb. A multiple regression of these and other chemical data suggested several factors had significant effects on accumulation. Seasonal effects were largely chemical in nature, rather than a function of the plants themselves.

Experiments supported several findings from the surveys. Zinc uptake proceeded more rapidly than loss and was influenced by aqueous Mg, Ca and humic acids, but not PO_4-P, NO_3-N or Si. Accumulation was greater in tips of Rhynchostegium than Amblystegium riparium or Fontinalis antipyretica.

Results indicate that bryophytes are useful as monitors of pollution. Rhynchostegium in particular is recommended for its ecological ubiquity, its presence in a wide range of aqueous metals and greater accumulation. Applications of bryophytes for specific uses are outlined, with recommendations for different situations. A new model, based on slopes of accumulation, is proposed as a predictive tool.

ABBREVIATIONS

s	second
min	minute
h	hour
nm	nannometre
μm	micrometre
mm	millimetre
cm	centimetre
m	metre
km	kilometre
μg	microgramme
mg	milligramme
g	gramme
meq	milliequivalent
ml	millilitre
l	litre
^oC	degrees Celsius
μS	microSiemen (= micromho)
μE	microEinstein
mCi	milliCurie
M	molar
EDTA	Ethlyenediamine tetraacetic acid
HA	humic acids
HEPES	N-2-hydroxypiperazine-N'-2-ethanesulphonic acid
FRP	filtrable reactive phosphate
FOP	filtrable organic phosphate
O.D.	optical density
r.p.m	revolutions per minute
cond	conductivity
U.V.	ultraviolet
n	number of measurements
$\bar{x}$	mean
SD	standard deviation
CV	coefficient of variation
p	probability
r	correlation coefficient
r^2	regression coefficient
Ho	null hypothesis
DOE	Department of the Environment

CONVENTIONS

flow	discharge of a stream or river
current	linear speed of a stream or river
soft water	waters with low total dissolved substances: esp. Ca < 50 mg l^{-1}
hard water	waters with high total dissolved substances: esp. Ca > 50 mg l^{-1}
Rhynchostegium	shortened name for *Rhynchostegium riparioides*

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CONTENTS

page

page

page

CHAPTER 7 EXPERIMENTAL STUDIES ON METAL ACCUMULATION BY AQUATIC BRYOPHYTES

CHAPTER 8 DISCUSSION

page

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1. INTRODUCTION

1.1 General Introduction

In recent years the combination of improved analytical techniques and increased interest in biological methods for surveillance has brought about a dramatic increase in the study of pollution (Cairns & Van der Schalie, 1980). There is a great variety of pollutants which affect inland waters and their complexity is frequently compounded when several organic and toxic pollutants enter a watercourse in combination, as from industrial or domestic effluents (Mason, 1981). This study considers principally heavy metals in streams and rivers, although such a study cannot ignore other man-induced perturbations, as well as the natural characteristics of freshwaters.

In this chapter an overview is given of the importance of heavy metals in the aquatic environment, with consideration to the nature and sources of these substances. Particular emphasis is directed towards the biological effects of heavy metals, with only general reference made to their geological and chemical importance. The biological assessment of water quality has developed historically around the study of organically polluted waters (Hynes, 1960; Mason, 1981) and more recent uses of biological methods for studying metal pollution are presented in section 1.3. Emphasis is given to the metal composition of organisms, especially aquatic bryophytes.

Bryophyte species of freshwaters are numerous, but since the paper by Watson (1919), no general discussion of the ecology of aquatic bryophytes has apparently been made. A summary of more recent research is therefore given (section 1.4), especially ecophysiological aspects. The general aim of these reviews (sections 1.2 - 1.4) is to emphasize the complementary positions that fundamental and applied research occupy in an interdisciplinary field. It is also the intention to establish what information is known, as well as where problems exist, from which the present study could continue. Some methodological studies have been conducted outside this thesis; thus an outline of the overall study is presented (section 1.5). Several findings have particular relevance to this study, and a summary of these results is given.

1.2 Heavy metals in freshwaters

Although used widely, the term "heavy metal," has never been clearly defined (Whitton & Say, 1975). Despite this ambiguity, heavy metals are usually considered (e.g. Passow et al., 1961; Lapedes, 1974) to include those metals with a specific gravity of five or greater. Nieboer and Richardson (1980) have argued on chemical and biological grounds that this term should be replaced with a classification of metals based on their relative affinity to oxygen or nitrogen/sulphur containing ligands. This is a more useful system to aid in the understanding of specific complexes, but can be cumbersome when dealing with environmental problems. Further, the term "heavy metal" remains in use in current textbooks on water chemistry (e.g. Ochai, 1977; Stumm & Morgan, 1981). Certainly there is room for both types of terminology, particularly when "heavy metal" has proved to be useful in arousing interest in these problems (Say & Whitton, 1981a).

The expansion of interest in heavy metals in aquatic environments is apparent when comparing earlier texts (e.g. Hynes, 1960; Wilbur, 1969) with more recent ones (e.g. Mason, 1981). Further information may be obtained from several recent reviews. A detailed treatment of the geochemistry of heavy metals in both marine and fresh waters has been given in the treatise by Förstner and Wittmann (1979). A diverse collection of reviews covering industrial, chemical, biological and medical aspects of heavy metals have been edited by Nriagu (1978, 1979, 1980a, b).

The biological effects of heavy metal pollution have been recognized for some time. One of the earliest studies on a river system was the series of papers on the softwater streams of Cardiganshire, west Wales (Carpenter, 1924), which were affected by old zinc and lead mines. Although most of the operations had ceased before studies began, these reports (summarized in Hynes, 1960; Whitton & Say, 1975) gave graphic accounts of the continued scarcity of plants and animals in rivers draining old mine workings (Rheidol and Ystwyth). The most affected streams had a sparse flora, with only a few bryophytes and the algae Batrachospermum and Lemanea. Animals which were common in adjacent unpolluted rivers, such as oligochaetes, leeches, molluscs and caddisflies were absent. It was recognized much later, however (Jones, 1940), that the toxic effects were probably due to zinc, rather than lead, in the water. A considerable pollution problem still exists to the present day (McLean & Jones, 1975). Similar long term effects have also been recognized in old lead-zinc mining regions of the Northern

Pennine Orefield in England (Say, 1977; Say & Whitton, 1981b). Mining (esp. Cu, Zn) has also been found to reduce the abundance and diversity of algae (Besch et al., 1972) and macrophytes (Besch & Roberts-Pichette, 1970) in the Northwest Miramichi River system, New Brunswick (Canada).

With such a clear picture of the continued detrimental effects that heavy metals can have on aquatic systems, it is of interest to consider the long term fate of streams draining recently developed mining operations, such as the "New lead Belt" of Missouri (Wixson, 1977). Some evidence already exists (Gale & Wixson, 1977) of reduced algal and invertebrate diversity in streams which receive effluents from mining activities. Biological effects may prove to be less severe than elsewhere, as pollution in the Missouri waters is at least partly ameliorated by naturally occurring hard water and elevated phosphate (Gale & Wixson, 1977).

Problems of metal pollution in freshwaters are not restricted to mining regions. Chronic copper pollution in the River Churnet (Trent catchment) from an industrial works (Pentelow & Butcher, 1938: cited in Hynes, 1960) was similarly devastating. Animals present above the effluent were absent for more than 15 km downstream. Algal growth immediately below the works was reduced by 90% and species composition was considerably less diverse. Heavy metal contamination may also result from other industrial sources, including the manufacture of paper, rubber, batteries, chemicals and paint (Mason, 1981). Intensive industrial development of the Mersey catchment has had a considerable effect since the industrial revolution (Harding, Say & Whitton, 1981). Heavy metals may also enter streams and their biota from highway runoff (Van Hassel et al., 1980), as a result of metal additives (esp. Ni, Pb) to automobile fuel. Metals in the form of radionuclides from nuclear reactors have also been identified in several rivers, including the Columbia in Washington, U.S.A. (Davis et al., 1958) and the Meuse in Belgium (Kirchmann & Lambinon, 1973).

While heavy metal contamination may bring about a reduction in the variety of many species (Hynes, 1960; Whitton & Say, 1975), it is not yet clear whether frequently used biological indices, such as the Trent biotic index or various diversity indices, will represent the effects of heavy metals as usefully as those of organic pollution (Mason, 1981), for which many of them were designed. The diversity of one community may respond differently to heavy metal pollution, compared with another. One example is the study of copper pollution in the River Churnet, mentioned earlier. As invertebrates were most severely affected, algal growth was able to increase at a site several kilometers below the outfall, because of the lack of grazing (Hynes, 1960). The abundance and variety of algae was considerable in this stretch, even though copper concentrations were still fairly high (0.6 mg l^{-1}). Further, there is certainly no agreed index among the great variety available (Whitton, 1979) and even less is known about their responsiveness to different types of pollution, particularly toxic substances such as heavy metals (Hawkes, 1982). Further discussion of the response and application of differences in species composition and community structure is given by Whitton (1979), Mason (1981) and Hawkes (1982).

1.3 Metal accumulation and monitoring

1.31 Scope

The primary aim of the present study was to examine the accumulation of metals by aquatic bryophytes, so most consideration will be given to accumulation by aquatic plants, particularly mosses and liverworts; brief mention of other organisms will also be made for comparison.

1.32 Metal accumulation in nature

Observations of the accumulation of metals from water by aquatic organisms in concentrations greater than those in the ambient environment are widespread; much of the literature is discussed elsewhere (Whitton & Say, 1975, Wong et al., 1978; Stokes, 1979). There are reports for algae from particularly diverse environments. Algal blooms from local ponds and marshes in the heavily industrialized lower Swansea valley were found (Trollope & Evans, 1979) to have elevated concentrations of iron, copper, zinc and lead, particularly blooms nearest zinc smelters. A tenfold difference was observed (Denny & Welsh, 1979) in lead concentrations in phytoplankton from Ullswater, English Lake District, which drains old lead mines, as compared with a small tarn lacking contamination. Elevated concentrations of lead were also measured in Ullswater zooplankton and the authors suggested that this was an indication of a transfer up the food chain. A trophic increase does not, however, appear to be as common for lead as it is for mercury (Wong et al., 1978). Cadmium could apparently be transferred up the food chain from contaminated fungal mycelia to Gammarus pulex, although there was no evidnce of biomagnification

(Duddridge & Wainwright, 1980).

Among algae sampled from streams for heavy metals, the red alga Lemanea (Kirchmann & Lambinon, 1973; Deb et al., 1974; Whitton & Say, 1975; Empain, 1976a; Empain et al., 1980; Harding & Whitton, 1981) and the green alga Cladophora (Gileva, 1960; Kirchmann & Lambinon, 1973; Gale & Wixson, 1977; Empain et al., 1980; Whitton, 1980; Lever, 1981) are the most common. Several of these studies have included bryophytes or other organisms and their results are discussed later. Heavy metal accumulation by estuarine and marine algae has been discussed in detail by Bryan (1969) and Phillips (1977, 1980).

Among the earliest reports of metal accumulation by aquatic bryophytes are those of Whitehead and Brooks (1969) and Brooks (1971) who used of these plants in geobotanical prospecting for minerals, particularly beryllium, copper, lead and uranium. This approach was based on the earlier use of terrestrial mosses and other plants as indicators, such as the so-called "copper-mosses," like Mielichhoferia (Hartman, 1969). Collections of bryophytes from rivers contaminated by radionuclides and heavy metals were soon widely reported (Dietz, 1973; Funk et al., 1973; Kirchmann & Lambinon, 1973; McLean & Jones, 1975; Whitton & Say, 1975; Empain, 1976a, b, 1977; Lloyd, 1977; Burton & Peterson, 1979a; Harding, 1980, 1981; Mouvet, 1980; Say et al., 1981, Wehr et al., 1981; Whitton et al., 1982; Say & Whitton, in press; Wehr & Whitton, 1983). Bryophytes most frequently sampled were the mosses Fontinalis antipyretica and Rhynchostegium (as Eurhynchium or Platyhypnidium) riparioides, and the leafy liverwort Scapania undulata. However, few of these studies were conducted outside Europe, where other species might have been sampled (section 1.42).

In those studies where several plants groups were sampled, bryophytes consistently accumulated heavy metals to a greater extent (on a dry weight basis) than did submerged shoots of angiosperms (Dietz, 1973; Kirchmann & Lambinon, 1973; Lloyd, 1977; Welsh & Denny, 1980). However, when bryophytes and algae have been collected from the same sites (Funk et al., 1973; Kirchmannn & Lambinon, 1973; Whitton & Say, 1975, Empain et al., 1980), differences between the two are not consistent and apparently vary with different elements. For example, Amblystegium cf. riparium (as A. juratzkanum) and Cladophora glomerata were collected from the upper Spokane River, Washington (Funk et al., 1973); the moss accumulated more manganese, iron and mercury, while the alga accumulated more copper, zinc, cadmium and lead. Comparisons between different species of bryophyte are also complicated, particularly because methods between various workers are far from standard (section 1.52). Say et al. (1981) found consistently greater concentrations of copper and cadmium in Rhynchostegium riparioides than Fontinalis antipyretica, using apical tips of moss, while concentrations of heavy metals (including Cu & Cd) in whole plants of these two species have been found to be very similar (Empain, 1976a, b).

A great variety of studies have reported the accumulation of metals by aquatic angiosperms, and only a mention of them is made here. General reviews include macrophytes (Wong et al., 1978; Stokes, 1979; Whitton, 1980) and, in particular, an extensive tabulation of results has been compiled by Dykyjova (1979). An important consideration when comparing results for aquatic vascular plants is that many species are rooted and may obtain metals both from sedimants and water (Harding & Whitton, 1978; Welsh & Denny, 1980); other submerged plants lack roots and are influenced primarily by aqueous metals (Dykyjova, 1979); still others have floating leaves or may even be partly emergent. Translocation may also affect element distribution in these plants (Welsh & Denny, 1979; Schierup & Larsen, 1981).

1.33 Factors affecting accumulation

Many factors are best illustrated using examples from several types types of aquatic plant. Many which have been suggested in field surveys and demonstrated in experiments appear to be general in nature and not restricted to particular plant groups.

The fact that many workers have now shown that significant positive correlations exist between aqueous and accumulated metals for a wide variety of plants (Stokes, 1979; Empain et al., 1980, Whitton 1980; Foster, 1982), suggests strongly that the amount of metal accumulated is primarily a function of the metal concentration in water. A number of studies (Gutknect, 1961, 1963; Pickering & Puia, 1969; Cushing & Rose, 1970; Wainwright & Beckett, 1975; Empain, 1977), using metabolic inhibitors, light/dark experiments or killed plants, have demonstrated that metal uptake is predominantly a passive, adsorptive phenomenon. Some scepticism must be reserved for studies using extreme methods for inactivating plant metabolism (e.g. DNP, heat or formalin), as these might alter ion exchange properties of the membrane. Indeed, Nakajima et al. (1981) have shown that the order of the selective uptake of nine metals by heat-killed Chlorella cells differed somewhat from live cells. This adsorptive uptake has also been shown to follow classic Langmuir kinetics (Pickering & Puia, 1969; Wainwright & Beckett, 1975; Empain, 1977), and the hyperbolic saturation curve is apparently universal among all organisms studies thus far.

Cations, including hydrogen (Gutknecht, 1961; Bachmann, 1963;

Francis & Rush, 1974; Wainwright & Beckett, 1975; Nieboer et al., 1976a, b; Stokes, 1979) and magnesium and/or calcium (Pickering & Lucas, 1962; Pickering & Puia, 1969; Cushing & Rose, 1970; Garland & Wilkins, 1981) may inhibit uptake of metals competitively. It has been argued (Bachmann, 1963; Ruhling & Tyler, 1970; Wainwright & Beckett, 1975; Nieboer et al., 1976a) that this is evidence of the ion-exchange quality which algae, lichens and bryophytes possess. Cadmium uptake by an alga (Nitella flexilis), an angiosperm (Elodea canadensis), a snail (Ampullaria paludosa) and a guppy (Lebistes retulatus) was significantly less in a regime with high (total c. 150 mg l^{-1}) combined calcium and magnesium (Kinkade & Erdman, 1975) than in a regime without these cations.

Effects of organic chelators in experimental media (e.g. EDTA), as well as those naturally present in river and lake water (e.g. humic acids: HA) have also been studied. Artificial chelators are known to form highly stable complexes with aqueous metals (Raspor et al., 1981; Stumm & Morgan, 1981) and can reduce uptake of iron (Geisy, 1976), copper (Stokes, 1979) and lead (Ahlf et al., 1980) by various freshwater algae. It has been well documented that HA can also bind metals such as manganese, iron, copper zinc and lead (Wilson, 1978; Kerndorff & Schnitzer, 1980), but few studies have examined their effects on metal accumulation. In one study (Geisy, 1976), iron uptake by Scenedesmus was reduced by the presence of HA.

Some of the most detailed experimental work on metal accumulation by aquatic mosses have been conducted using radioisotopes. A series of studies on Rhynchostegium (Platyhypnidium) riparioïdes by Hébrard and coworkers (Hébrard et al., 1968; Hébrard & Foulquier, 1975; Foulquier & Hébrard, 1976) examined rates of uptake and loss, sediment interactions, localization and the exchangeability of several elements. Metal uptake was rapid, usually reaching equilibrium in less than 10 h, but the total quantities adsorbed were reduced when sediments were present. Desorption in an uncontaminated medium proceeded more slowly than uptake, and a proportion of the total accumulated metal remained bound even after several days. More metal was released in the presence of uncontaminated sediments. There was no evidence for the uptake of metals by aquatic mosses directly from sediments. However, their medium, which contained fairly high magnesium (29 mg l^{-1}) and calcium (76 mg l^{-1}) concentrations,may have inhibited uptake to some degree. An interesting technical point arising from this work is that container walls were also capable of adsorbing 50% of the metals in the absence of moss and sediment. When sediments and mosses were both present, loss was still as great as 10%.

One subject which has attracted considerable interest is the relationship between tolerance to metals and accumulation. Perhaps the greatest progress has come from work with terrestrial plants (Antonovics _et al._, 1971; Baker, 1981) which are capable of colonizing metalliferous soils. Reports for algae (Foster, 1977; 1982; Butler _et al._, 1980) have shown that populations from metal-rich environments may achieve a degree of tolerance through exclusion of metal ions, and thus reduce toxicity. However, greater uptake of metals in one tolerant algal population, as compared with its non-tolerant counterpart, has also been reported (Hutchinson & Stokes, 1975). _Lemanea_ populations from unpolluted and zinc-polluted sites accumulated zinc to similar concentrations, when both were transplanted into a stream with high concentrations (> 1.0 mg l^{-1}) of zinc (Harding & Whitton, 1981).

Although no interpopulation comparisons are known for aquatic bryophytes, uptake of ^{65}Zn by Fontinalis squamosa, which was presumed non-tolerant, was found to be less than for Scapania undulata, which was presumed to be tolerant to heavy metals (McLean & Jones, 1975). No consideration was made, however, for interspecific differences between the mosses, such as morphology, size or weight. A comparison (Brown & Bates, 1972) between tolerant and non-tolerant populations of a terrestrial moss, Grimmia donniana, showed no significant difference in lead uptake between the two.

1.34 Monitoring

Methods for the use of biological monitors have been discussed by Phillips (1977), Stokes (1979) and Whitton (1980) and the relative merits of each compared. The method discussed here is based on the accumulation of metals and the measurement of the concentrations of these metals in aquatic plants. The term "indicator" has also been used in this context, but may lead to confusion as it has also been used in situations (not all with heavy metals) where simply the presence or absence of pollution tolerant (e.g. Stigeoclonium, sewage-fungus) or sensitive (e.g. Cladophora, some stoneflies) species is thought to indicate water quality (Hynes, 1960; Wilhm, 1975; Whitton, 1980).

According to Empain and coworkers (Empain 1976a,b; Empain et al., 1980) and Phillips (1977), biological monitors may be recommended for the following reasons:

1. aquatic plants accumulate metals which allow simple and inexpensive analysis;
2. metals accumulated are an indication of their biological availability at a particular site;
3. plants act as integrators of conditions, thus results provide a moving average of aqueous metal concentrations.

Plants have obvious advantages over water, which is more laborious and expensive for analysis and requires multiple samples to account for variation in time. Sediments also are less useful, as differences in local sedimentation rates and their mobility affect results (Phillips, 1977).

Among those applied studies which have used aquatic plants, reports for algae (Timofeev-Resoviskii et al., 1960; Keeney et al., 1976; Johnson et al., 1978; Ahlf & Weber, 1981; Lever, 1981), bryophytes (Kirchmann & Lambinon, 1973; Empain, 1976a, b; Mouvet, 1980; Harding, 1981; Say et al., 1981) and angiosperms (Ray & White, 1976; Mudroch & Copbiancio, 1979; Nakada et al., 1979; Aulio, 1980; Franzin & McFarlane, 1980; Jahnke et al., 1981; Lever, 1981) are known. In all cases, the accumulation of metals in plants collected from sites affected by a particular pollutant was observed. In not all cases, however, were samples also taken from sites unaffected by heavy metals, so it is difficult in these cases to state quantitatively how much enrichment there was.

Comparisons of enrichment are complicated simply because aqueous concentrations differ. One index which accounts for these differences is the enrichment ratio. This is defined as the concentration of a metal in an organism divided by the aqueous concentration (Whitton & Say, 1975). The index has been used widely (under various names), but has been criticized (Jinks & Eisenbud, 1972; Kneip & Lauer, 1973; Whitton & Say, 1975) for its variability between different species and environments. This perhaps is not surprising, in light of the factors discussed earlier which influence metal accumulation. At least among aquatic bryophytes, some of this variability is certainly due to analytical and methodological differences as well (section 1.52).

One profitable byproduct which has emerged is the use of aquatic plants to remove heavy metals from effluents by accumulation, prior to the discharge into rivers or lakes (Rana & Kumar, 1975; Filip et al., 1979; Gale & Wixson, 1979). Mostly algae have been grown in these effluents, although mosses are known to occur naturally in many old mine discharges (Say & Whitton, 1981b). Information on the relative differences in accumulation by various plants from monitoring and fundamental studies would be useful in this context.

1.4 Ecology of aquatic bryophytes

1.41 Introduction

Bryophytes are widespread in aquatic environments, particularly flowing waters, and in some habitats, may be the dominant aquatic plants. Several mosses and liverworts are predominantly or wholly aquatic species (Watson, 1919; Crum & Anderson, 1981). However, among botanists studying the flora of rivers (e.g. Haslam, 1978, 1982a, b) and bryophyte ecologists (e.g. Longton, 1980; Smith, 1982), aquatic bryophytes have received less attention than might be expected from their relative importance in many freshwater systems.

1.42 Distribution

1.421 Geographic distribution

Several genera are widely reported from aquatic habitats (Table (Table 1.01). Some are predominantly aquatic, while others, such as *Brachythecium* and *Bryum*, have representatives in both terrestrial and aquatic habitats.

Perhaps the most widely known aquatic moss is *Fontinalis*, which is common world-wide and is more often recognized in macrophyte surveys by workers studying higher plants. This is a cosmopolitan genus whose species are more restricted. *F. antipyretica* and *F. squamosa*, are the most common in European rivers (Warnstorf *et al.*, 1914; Nyholm 1954-1969; Smith, 1978), but in North America, *F. dalecarlica*, *F. novae-angliae* and *F. neo-mexicana* are common (Welch, 1960; Glime, 1968; Crum & Anderson, 1981). Roughly two-thirds (730 in total) of 1055 1-km

Table 1.01. Examples of common genera of aquatic bryophytes from lotic environments (based on Watson, 1919; Glime, 1968 and Watson, 1968; nomenclature follows Holmes *et al.*, 1978 and Smith, 1978).

mosses	liverworts
Amblystegium *	*Chiloscyphus*
Brachythecium	*Cepahalozia*
Bryum	*Jungermannia*
Cratoneuron	*Nardia*
Cinclodotus	*Riccardia*
Fissidens	*Riccia*
Fontinalis	*Scapania*
Hygrohypnum	*Solenostoma*
Rhynchostegium **	

* some authors use *Leptodictyum*
** several names used: see section 2.72

lengths, representing 130 rivers surveyed in England, Scotland and Wales by N.T.H. Holmes (pers. comm.) had F. antipyretica present. This and Rhynchostegium riparioides (present in 729 sites) were the two most frequently encountered bryophytes.

Welch (1960) notes that until its introduction in South Africa, Fontinalis antipyretica was unknown in the southern hemisphere. The spread of this "exotic" in the Eerste River brought about a marked change in the microhabitat of invertebrates that previously fed on mucilaginous algae (Richards, 1947). Several bryophytes which have been reported (Craw, 1976) in streams of New Zealand (e.g. Fissidens rigidulus, Monoclea forsteri) are uncommon or unknown in Europe (Smith, 1978) and North America (Crum & Anderson, 1981). Some aquatic mosses have an even more restricted distribution, such as Donrichardsia macroneuron, which is a monotypic genus (family Amblystegiaceae) known only from a few limestone springs in the Edwards Plateau, Texas (Wyatt & Stoneburner, 1980).

Although discontinuities in distribution may occur, other species common in European rivers are widespread. For example, Rhynchostegium riparioides is known from places as distant and varied as Iceland, Lebanon, Tibet, China and North and South America; Amblystegium riparium occurs in Siberia, Japan, North America, Australia and Southern Africa (Smith, 1978; Crum & Anderson, 1981). It is tempting to suggest that the distribution of these two species, both of which regularly produce sporophytes, may have been aided by spore dispersal, which occurs only rarely in Fontinalis antipyretica (Smith, 1978; Glime et al., 1979).

1.422 Ecological distribution

Bryophytes are found in a great variety of freshwater habitats, from small springs and headwater streams to ponds and large lakes (Welch, 1960; Watson, 1968; Hynes, 1970; Crum & Anderson, 1981). The longitudinal distribution of bryophytes and other macrophytes within a river has been studied extensively. Several authors (e.g. Sirjola, 1969; Dawson, 1973; Holmes & Whitton, 1977b, 1981) have observed a zonation of macrophyte vegetation in rivers, with a dominance of bryophytes in the upper zone, where typically the substratum consists of large rocks and current velocity is high. Zonation may be a misleading term in that it implies distinct communities of plants, since more often a complex gradient is actually the case (Westlake, 1975).

Certain bryophytes also display preferences for particular types or sections of river. In a New Hampshire stream, upstream sites were found (Glime, 1970) to be colonized predominantly by Scapania undulata and S. nemorosa, which were replaced by Fontinalis novae-angliae and Plagiochila asplenoides further downstream. Several major rivers of north-east England (Holmes & Whitton, 1975a, b, 1977a, b, c, 1981), the River Wye on the England - Wales border (Merry et al., 1981) and the River Etherow, Greater Manchester (Harding, Say & Whitton, 1981) have been studied. In these, the liverworts Chiloscyphus polyanthos and Scapania undulata, and the mosses Hygrohypnum ochraceum and H. luridum were common in the upper reaches. Amblystegium riparium and Fontinalis antipyretica were found further downstream. Cinclidotus fontinaloides and Rhynchostegium riparioides were commonly found along a great proportion of a river's length. Fontinalis sqamosa was widely distributed in rivers from western Britain only. It is also reported in Wales

(McLean & Jones, 1975). This is given special comment below.

Based on observations (the author, unpublished) of herbarium records and specimens from the Hancock Museum (Newcastle upon Tyne), Fontinalis squamosa was sampled from a lowland reach of the River Wear near Chester-le-Street (County Durham) prior to 1805 (see also Winch et al., 1805) and as recently as 1930 (collectons of M. Dalby, unpublished). The apparent absence of this species nowadays in the Wear (Holmes & Whitton, 1977c) and probably elsewhere in north-east England (N. T. H. Holmes, pers. comm.) suggests some change in conditions in the Wear, which has not also occurred in other rivers such as the Etherow. It has been observed (Holmes & Whitton, 1977a) that three macrophytes apparently "disappeared" from the River Tees within a period of five years, hence caution should be exercised when attempting to explain long-term changes in distribution.

Distribution patterns in aquatic bryophytes have also been observed on a smaller scale. The vertical distribution of bryophytes along streamside rocks and cliffs can be quite marked (Gimingham & Birse, 1957). This community has been observed to be composed of as many as 18 different species (Craw, 1976), distributed along a steep moisture gradient. Associations of sympatric Cinclidotus species apparently separate similarly (Lambinon & Empain, 1973). Microhabitats differences in current velocity can also separate species. Fissidens rigidulus was considerably more abundant in torrential water in one New Zealand stream than other mosses (Cowie & Winterbourn, 1979). On individual boulders in a Swedish stream, Hygrohypnum ochraceum was observed (Johnson, 1978) to colonize consistently the upper surfaces in the strike of the current, while Fontinalis dalecarlica was restricted to the lee sides. The Rheinfalls (River Rhein, Switzerland) are covered almost entirely by Cinclidotus danubicus and Rhynchostegium riparioides (Jaag, 1938). Other species show similar patterns of microdistribution in smaller streams (Jovet, 1932; Tutin, 1949; Matoničkin & Pavletić, 1961).

Spatial distribution of bryophytes in lakes is apparently related primarily to depth. Some species have been observed to great depths (Hiltunen, 1966; Bodin & Nauwerck, 1968; Light & Heywood, 1973; Light, 1975; Priddle, 1980a). A remarkable collection of Amblystegium riparium, typically a moss of organically polluted rivers and sewage works (Cooke, 1953; Hussey, 1982), was made from "ultraoligotrophic" Lake Tahoe (Franzin & Cordone, 1967) at depths from 35 - 120 m. As many as 13 aquatic bryophytes were identified from samples taken by dredging strictly between depths of 35 - 150 m. An interesting picture of the depth distribution of bryophytes in Scottish lochs has been described (Light & Lewis Smith, 1976). One depth transect in Loch Einich discovered nine species from the 1 - 2 m depth; five at 5 - 8 m; but only one (".... a tall, robust form of Eurhynchium (=Rhynchostegium riparioides") at 12 m. Nearly 40% of the bottom of one deepwater lake in Sweden has been found to be covered by Marsupella aquatica (Bodin & Nauwerck, 1968), with the largest plants at the 25 - 30 m depth.

1.43 Factors affecting aquatic bryophytes

1.431 Importance of environmental factors

A consideration of distribution patterns both geographically and in microhabitat, suggests factors which may influence the presence or abundance of an aquatic bryophyte. Such influences may also be more subtle and affect the physiological state or morphology of a particular plant. The choice of which factors are studied is in part subjective and can be complicated, particularly in flowing waters where environmental variables are often interrelated. Therefore, some of the early descriptive literature are considered tentatively, primarily because much of it suffers from a lack of experimental data to substantiate otherwise careful observations.

1.432 Physical factors

Temperature

Temperature variation in rivers can be considerable, both seasonally and diurnally (Hynes, 1970). Apparently, freezing is not permanently damaging to most non-aquatic bryophytes (Longton, 1980). An abundance of bryophytes have been reported (Priddle & Dartnall, 1978; Priddle, 1979) on lake bottoms in Antarctica, which freeze to about the 1 m depth. However, maximum development of these plants were at greater depths. Low temperatures can also have indirect effects. All but the shallows of Lake Latnajajaure (Sweden) were colonized by one hepatic, due to the mechanical effects of ice in the upper 2 m (Bodin & Nauwerck, 1968). The occurence of bryophytes in the opposite extreme appear to be rare, although Richards (1932) has observed that _Archidium alternifolium_, which occurs in marshes and ditches in southern U.S.A., the Canary Islands and south-central Europe (Crum & Anderson, 1981), is able to extend its range into sites in Iceland by colonizing the margins of streams receiving thermal springs. A species of _Fontinalis_ has been observed (the author, unpublished) growing abundantly in Firehole River, below the geysers. Summer temperatures typically reach 30°C in the summer, although micro-regimes may be considerably warmer (Castenholz & Wickstrom, 1975).

Growth of *Amblystegium riparium* has been shown (Sanford, 1979) to increase over the range 5 - 22 $^{\circ}$C, but decline at higher temperatures. Over two years, a nearly perfect correlation was found (Johnson, 1978) between the seasonal fluctuation in river water temperature and growth in a population of *Fontinalis dalecarlica*. Temperature increases have been shown experimentally to cause an increase in rhizoid production by *F. hypnoides* and *F. novae-angliae* (Glime, 1980). A comparison (Empain, 1977) of growth rates in eight aquatic mosses over a range of temperatures from 8 - 25 $^{\circ}$C found that under lower light, most species reached a maximum between 10 - 15 $^{\circ}$C, although in *Rhynchostegium riparioides* the growth rate was still increasing at 25 $^{\circ}$C. An interaction was observed between temperature and light, with many species tolerating higher temeratures under increased irradiance.

Temperature effects apparently must be considered in relation to to the conditions from which a bryophyte is isolated. Winter acclimated *Fontinalis novae-angliae* was shown (Glime & Carr, 1974) to lose colouration and in some cases leaves as well, at temperatures greater than 15 $^{\circ}$C. Nonetheless, plants were capable of survival even after being submerged in 85 $^{\circ}$C water continuously for four days.

Degree of submergence

After observing differences in the frequency of emersion between different moss species in the Belgian section of the River Somme, Empain (1977) designed experiments which compared the growth of eight mosses in the laboratory over different frequencies of emersion, from 0 to 99.8%. *Amblystegium fluviatile* and *Rhynchostegium riparioides*, which were commonly observed over a broad vertical range in nature, had no significant difference in growth relative to emersion frequency in a 15 day period. Growth of Fontinalis antipyretica was reduced by roughly two-thirds when emersed 99.8% of the time. Leaf size in Calligeron sarmentosum is considerably smaller in emergent populations than submerged ones and when grown submerged will adopt the larger leaf size (Priddle, 1979). Scapania undulata (Gupta, 1977) and Fontinalis antipyretica (Brown & Buck, 1979) both show greater signs of membrane damage from desiccation (leakage of intracellular ions or organic solutes) than non-aquatic bryophytes. Another aquatic, Hygrohypnum luridum, was unable to resume protein synthesis when artificially desiccated (Bewley, 1974). This was apparently irreversible even after 20 h rehydration.

Although aquatic bryophytes clearly undergo considerable damage during desiccation, many mosses and liverworts experience and tolerate extended periods of emersion during low flows in rivers. In a simple but elegant experiment, Glime (1971) removed from a river a series of boulders which were covered with Fontinalis dalecarlica or F. novae-angliae and placed them on the bank well above the maximum water level for periods of up to eight months. Although most plants appeared chlorotic or even brown, both species regained a normal green colour within several days of their reintroduction into the stream. Atmospheric humidity was apparently sufficient to permit their survival, as a parallel set of plants died within 55 h in the laboratory.

Current velocity

Earlier reference to the microdistribution relative to the exposure to current suggests species may have different optima. Watson (1919) recognized more than 50 years ago that several mosses are more common in slowly flowing rivers than others. It has been found (Sirjola, 1969; Dawson, 1973; Johnson, 1978) in several streams that bryophytes tend to predominate over other plants in areas of greatest current velocity. This has been suggested to be related to greater availability of CO_2 (Hynes, 1970; see also section 1.433), although experimental studies *in situ* are lacking.

Current can also act to reduce accumulation of detritus and aufwuchs (fungi & algae) on bryophytes (Johnson, 1978). Aquatic mosses have been shown to adapt to increased current velocity by increasing production of rhizoids and rhizoidal branches for attachment (Glime, 1980). Although an innate, physiological demand for increased current velocity has been demonstrated for several lotic algae (Hynes, 1970), this response has yet to be shown for bryophytes.

Light

Earlier comments (section 1.432) on Empain's (1977) experiments on growth rates made reference to the fact that the effects of light may interact with temperature. This is probably true for many factors, as suggested at the beginning of this section. Perhaps the greatest amount of work on the effects of light have been in relation to productivity. At temperatures above 5 ^{o}C, a considerably greater irradiance was found necessary (Priddle, 1980b) to keep productivity of mosses in Antarctic lakes above the compensation point. Interestingly, for *Drepanocladus* cf. *aduncus*, this was nearly five times lower than that for *Calligeron sarmentosum*, which may explain why the former is capable of living at greater depths. Deep water populations of *Marsupella aquatica* are capable of nearly constant production and assimilation rates over a range of more than two orders of magnitude difference in irradiance (Bodin & Nauwerck, 1968).

Pickering and Puia (1969) have demonstrated that the rapid adsorption of ^{65}Zn by Fontinalis antipyretica was the same whether it took place in the light or in the dark. It was shown that prior to any active, membrane-related processes, a passive adsorption occurred. A slower active stage across the membrane followed, which was light dependent. It has been demonstrated with the liverwort Riccia fluitans (Felle & Bentrup, 1976) that in the light, the flux of ions across the membrane was active and governed by electromotive forces, while in the dark it was governed largely by diffusion.

Other physical factors

Aquatic bryophytes are typically found in rivers attached to solid substrates, including stones, logs, and nearly any stable inanimate object. It has been demonstrated (Glime et al., 1979) that the rate of rhizoidal attachment is influenced by the type of substrate available. Attachment was least efficient on hard, nonporous rocks like granite and was most successful on sandstone. However, mosses have even been observed (Neumann & Vidrine, 1978) growing on live freshwater mussels. The need for a hard, stable substrate is presumably less important in lakes, where plants are reported on sand and organic mud (Bodin & Nauwerck, 1968; Light & Lewis Smith, 1976). It would be interesting to know whether such plants still produce rhizoids.

A feature common to many rivers is the presence of suspended matter. It is thought the primary effect on plants is the reduction of light available for photosynthesis (Hynes, 1970). However, suspended coal particles which are discharged into rivers have been shown (Lewis, 1973a, b) to affect the growth of Rhynchostegium (as Euryhynchium) riparioides through physical damage and not through the reduction of light.

1.433 Water chemistry

Dissolved gases

Perhaps more physiological studies on aquatic mosses and other macrophytes have been conducted on the properties of CO_2 and HCO_3^- utilization than on any other subject. Early studies (Ruttner, 1947; 1948) found that several bryophytes were unable to take up bicarbonate for photosynthesis, led to the explanation that these plants were restricted to rapid water where turbulence would reduce CO_2 limitation and minimize the boundary layer near the cell surface (Bain & Proctor, 1980; Smith & Walker, 1980). This is at first surprising, because some mosses, such as Fontinalis antipyretica are known to occur in rivers with reduced current velocity and higher pH, where HCO_3^- is the predominant inorganic carbon source. Bain & Proctor (1980) have suggested that in such situations CO_2 depletion during photosynthesis results in a bicarbonate gradient forming around the plants; CO_2 diffusion is thus supplemented by this gradient.

pH

Athough it has been reported (Glime, 1968; Hynes, 1970) that certain species, such as Fontinalis squamosa and Scapania undulata predominate in softwater streams with lower pH values and species such as F. antipyretica occur primarily in waters of higher pH, there is little direct evidence of limitation specifically due to pH. This is primarily because pH can act to influence the $CO_2 - HCO_3^-$ eqilibrium, as well as the solubility of other ions (Stumm & Morgan, 1981). For example, the lower pH (4 - 7) waters common for Scapania (Whitton et al., 1982) frequently have low aqueous calcium. Potential competitors which cannot tolerate calcium-poor streams would be absent. A reduction in photosynthesis over the pH range 7.2 - 8.2 in Cinclidotus nigricans was probably the result of the reduced CO_2 availablity (Empain, 1977). In waters with very low pH (< 4.0), species composition changes markedly and bryophyte species such as Drepanocladus fluitans (Whitton & Say, 1975), Dicranella heteromalla or Cephalozia bicuspidata (Wehr & Whitton, 1983) may predominate.

Nutrients and metals

The effects of nutrients are difficult to demonstrate in field studies alone (Westlake, 1975). In batch culture, growth of Rhynchostegium riparioides shoots was significantly increased (Lewis, 1974) by greater phosphate (1.0 - 40 mg l^{-1}), but not by ammonia, nitrite or nitrate (< 0.5 - 500 mg l^{-1} -N). Frahm (1975) has tested the "tolerance" of five aquatic mosses to several ions common in organic pollution. In all cases, Amblystegium riparium tolerated concentrations as great or greater than all other species (including Fontinalis antipyretica & Fissidens crassipes); this was as much as 25 mg l^{-1} PO_4 -P and 1550 mg l^{-1} Cl. Organic additions (e.g. sugars) to an inorganic medium can apparently induce sporophyte production (Belkengren, 1962) in Amblystegium riparium. This may help to explain the success of this moss in organically polluted waters. It was also found, however, that those factors which were successful at promoting sexual reproduction led to a reduction in leafy growth.

Based on quantitative samples of 22 bryophyte species and physicochemical variables from rivers in the Meuse and Somme basins in Belgium, Empain (1978) summarized the "resistance" of these species using multivariate techniques. An index was developed from these results according to the degree of pollution (including organics and heavy metals), and the moss identified as most tolerant overall was Rhynchostegium (Platyhypnidium) riparioides. Individual pollutants may have different effects, however. Copper has been shown to be more inhibitory to shoot production in R. riparioides than are manganese, iron, zinc and lead (Lewis, 1974). Cadmium has been shown (Sommer & Winkler, 1982) to reduce photosynthesis by Fontinalis antipyretica more effectively than equivalent amounts of copper at concentrations up to 100 mg l^{-1}; above this copper was more inhibitory. Nonetheless, several bryophytes do occur in waters with fairly high concentrations of metals (e.g. > 1.0 mg l^{-1} Zn), including Bryum pallens, Dicranella varia and Scapania undulata (Say & Whitton, 1981b); species in streams with somewhat lower zinc concentrations include Brachythecium rivulare, Hygrohypnum ochraceum and Rhynchostegium riparioides.

The occurrence of bryophytes in a wide range of chemical conditions is also demonstrated by the fact that several species can occur in streams in association with travertine formation (Jovet, 1932; Matoničkin & Pavletić, 1961; Pentecost, 1982). Two species present in all three of these springs were Barbula tophacea and Rhynchostegium riaprioides, although neither were dominant species. Cratoneuron commutatum was in fact most abundant in two of them. Matoničkin and Pavletić (1961) point out that species may still separate ecologically within such a specialized environment, as, for instance with respect to current velocity; in this study the amount of tufa deposition was itself related to local variations in current velocity. This is one example where it is difficult and perhaps not realistic to isolate a single factor as the cause of microdistribution patterns.

1.434 Biological factors

Although most river macrophytes are not grazed to the same degree as algae (Westlake, 1975), some invertebrates, including caddisflies (e.g. Crunoecia) and mayflies (e.g. Ephemerella), do feed primarily on aquatic bryophytes (Percival & Whitehead, 1929). Several other animals which feed on detritus or algae (e.g. stoneflies) may also feed on mosses, though to a lesser extent. Several caddisflies (Hydroptilidae) construct cases out of aquatic bryophytes and some use only one particular moss or liverwort, even when more than one is available (Glime, 1978).

Biological interactions are not limited to herbivory. Prior (1966) has isolated a population of Amblystegium riparium which was parasitized by the fungus Stemphyllium botryosum. Fungal hyphae penetrated the stem and leaves of the moss and produced conidia. This was diagnosed as a true obligate parasite and the infected moss gametophyte became obviously stunted and misshapen.

1.44 Importance of bryophytes in streams

As bryophytes are the dominant aquatic vegetation in sections of rivers such as the Rhein (Jaag, 1938), Meuse, Somme, (Empain, 1978) and Tees (Holmes & Whitton, 1981), it might be assumed that these plants are responsible for the majority of the primary production in some large rivers. However, there is direct evidence for this only in small streams (Dawson, 1973).

Aquatic bryophytes, along with sediments, can function as "buffers" of phosphate concentrations in streams by their rapid uptake of phosphate released from leaf breakdown or even anthropogenic sources (Meyer, 1979). The importance of two species of Sphagnum in regulating metal concentrations (Na, Mg, K, Ca, Fe) in a small stream has also been demonstrated (Bell, 1959). Plants collected from the stream were placed in a column and cations were stripped from dilute solutions when passed across the (still live) plants. Some metals were removed from solution selectively (esp. Fe) and the pH was depressed through the exchange of hydrogen ions.

1.5 Practical organization of the overall study

1.51 Administration

The overall project, of which this thesis is a part, has been a joint effort with Dr. B. A. Whitton and Dr P. J. Say, under a contract from the Department of the Environment (DOE), U.K., to develop methods for the use of bryophytes as monitors of heavy metals in rivers. This was later expanded to include other aquatic plants, but the primary emphasis remained with bryophytes. The project benefitted from collaboration with other workers, particularly Water Authority biologists (esp. Dr J.P.C. Harding[1], Northwest Water Authority, Warrington) and other researchers (esp. Dr A. Empain[2] & C. Mouvet[3], University of Liège, Belgium).

Research on the contract began with general surveys of metal accumulation by aquatic bryophytes and case studies in specific rivers where heavy metal pollution was suspected. Further work compared approaches and methods which have been in use by other workers. Detailed studies were then carried out along three lines: 1) examination of specific factors which are of importance to metal accumulation; 2) comparisons of analytical methods and 3) consideration of the quantitative and statistical problems relating to such studies. In this thesis the first and third points have been given the greatest consideration, while analytical studies were carried out jointly.

1. presently at Severn-Trent Water Authority, Soar Division, Leics.
2. presently at Jardin de Botanique, Miese, Belgium.
3. presently at University of Metz, France.

1.52 Outline of joint studies

Several projects completed under this contract are already published or are in preparation for publication. An outline is given here, including those in which the present author was not directly involved (Table 1.02).

In a review of the applications and advantages of the use of plants as monitors, Whitton et al. (1981b) have emphasized that choice of a specific approach is influenced by the predictability and the type of pollution, as well as the needs of the particular water management body or researcher. Methods used in Durham for sampling, storage and processing aquatic bryophytes have in large part followed methods used by Say et al. (1981), in a study of metal pollution in the River Etherow, Greater Manchester. Full details are given in the following chapter (section 2.24), but can be summarized briefly in five steps: 1) collection of byophytes from rivers in areas of greatest current velocity, with preliminary washing in river water; 2) storage of fresh material over short-term in moist, chilled condition; 3) further washing in several changes of deionized water in laboratory; 4) use of apical tips of moss as the fraction for analysis; 5) rapid drying of moss prior to digestion.

Later, comparisons were made (Wehr et al., in press) with methods developed by Empain and coworkers in Liège, Belgium (Empain, 1976a, b, Empain et al., 1980; Mouvet, 1980), which revealed advantages of both approaches. A brief summary of the findings is given here. Storage of air-dried packets of moss after collection (Liège methods) is considerably easier and less costly than storage of fresh material, while concentrations of some metals were slightly (but significantly) greater in mosses processed by the former method. A considerable amount

Table 1.02. Studies and reports carried out under contract from the Department of Environment (BAW = B.A. Whitton, PJS = P.J. Say, JDW = J.D. Wehr, + = others in collaboration; * = some species not yet organized into reports).

project	status	persons involved	reference in thesis
I. <u>Case studies</u>			
1) Cr & Zn pollution, R. Etherow	published	PJS, BAW, +	Say *et al.* (1981)
2) Plants & animals of R. Etherow	published	PJS, BAW, +	Harding, Say & Whitton (1981)
3) Cr pollution, R. Holme	in prep.	PJS, BAW, +	
4) Zn pollution, R. Team	published	JDW, PJS, BAW	Wehr *et al.* (1981)
II. <u>Surveys</u> *			
1) Use of *Scapania unduluta*	published	BAW, PJS, +	Whitton *et al.* (1982)
2) Use of *Fontinalis antipyretica*	in press	PJS, BAW	Say & Whitton, (in press)
3) Use of *Rhynchostegium riparioides*	Ph.D.	JDW	
4) comparison of moss species	Ph.D.	JDW	
III. <u>Methods</u>			
1) Processing methods	in press	JDW, PJS, BAW, +	Wehr *et al.* in press
2) Digestion methods	in prep.	JDW, PJS, BAW	
3) X-ray fluorescence	published	PJS, BAW, +	Satake *et al.* (1981)
IV. <u>General</u>			
1) Review of plants as monitors	published	BAW, PJS, JDW	Whitton *et al.* (1981b)
2) Plants & accumulation in acid spring	published	JDW, BAW	Wehr & Whitton (1983)

of time may be spent in washing bryophytes to remove sediment and debris (Burton & Peterson, 1979a; Wehr et al., in press). Recent work shows clearly that a vigorous multiple compartment washing system (Liège) can process considerably greater amounts of material per unit time, but some loss of metals results. Therefore, the scale and needs of the particular study must be considered.

Whole plants (Liège) were found to contain significantly greater concentrations of metals than apical tips, but with some metals the variation in results was considerably greater. It was believed that apical tips were more responsive to fluctuating or unpredictable pulses of aqueous metals, but no direct comparisons had yet been made.

Rapid drying at a fairly high temperature (Durham) was felt to be advantageous because a constant dry weight is obtained without encouraging plant degradation or bacterial growth. The temperature chosen was 105 $^{\circ}$C, which is used widely for ecological materials (Allen, 1974). Empain and coworkers use 40 $^{\circ}$C as a more "natural" temperature, based on microthermister measurements of emergent moss clumps in the field (Empain, 1977). Temperatures used by others also include 20 $^{\circ}$C (Ruhling & Tyler, 1970), 70 $^{\circ}$C (Foster, 1982), 80 $^{\circ}$C (Burton & Peterson, 1979a) and 90 $^{\circ}$C (Hutchinson & Stokes, 1975; Welsh & Denny, 1980). The majority of studies pertaining to metals in organisms apparently use 105 $^{\circ}$C. At 40 $^{\circ}$C, mosses were found to be dried incompletely even after several days, which resulted in an overestimate of plant weight. This in turn gave "lower" metal concentrations, when expressed on a dry weight basis (Wehr et al., in press). No evidence has been found, for the metals studied (Ca, Mn, Fe, Ni, Co, Cu, Zn, Cd, Pb) of losses due to volatility at 105 ^{o}C, although it would presumably be a consideration if mercury were to be analysed.

Procedures used by various workers for the acid digestion of biological material (see studies cited this chapter) are perhaps the most varied and confusing of all analytical methods used. The variety of methods are in fact nearly as diverse as the studies themselves. One characteristic common to many studies is the apparent requirement for a complete destruction of organic material in order to elute metals completely. Frequently this has involved the use of mixtures of concentrated acids, including perchloric (e.g. Ruhling & Tyler, 1970; Empain, 1976a, b; Baker, 1978; Williamson, 1979). During studies on storage and processing (Wehr et al., in press) it was found that without using acid-matched standards, concentrations of certain metals (esp. Ca, Ni, Zn), as measured by atomic absorption, were suppressed. Subsequently, the efficiency of several concentrated acids (nitric, sulphuric, perchloric, two- and three acid mixtures, plus other oxidizing agents) and a series of dilute acids were evaluated. Significantly greater metal concentrations were measured in digests of two mosses (Fontinalis, Rhynchostegium) using more dilute nitric acid (between 1 - 5 M). Subsequent studies on the alga Cladophora (I. G. Burrows, pers. comm.) and several aquatic invertebrates (J. A. Palmer, pers. comm.) have found similar results. A few workers (Brown & Bates, 1972; Levy & Cromroy, 1973; Puckett et al., 1973; Brooks & Crooks, 1980) have satisfactorily used dilute (usually 1 - 2 M) acids in their studies. Results conducted in Durham suggest convincingly that the complete oxidation of organic matter is unnecessary for efficient elution of metals from a range of biological materials and in many cases the more dilute acids perform significantly better. Following these results, and in light of the greater backround contamination, hazard and cost of concentrated acids, the use of 2 M nitric acid has been adopted for digestion of plant material in Durham.

1.6 Objectives

The review of the pertinent literature (sections 1.2 - 1.4) and the overview of some of the current joint studies on methodology and case studies have revealed several areas worthy of further study. A considerable amount of both applied and quite fundamental work has already been carried out on metal accumulation (section 1.3), but few have tried to integrate these two aproaches into one study. Therefore the specific aims of this study were decided to be the following:

1) To examine, by the use of thorough field surveys, the extent of heavy metal pollution in streams and rivers where aquatic bryophytes occur.

2) To compare the accumulation of metals by field populations of bryophytes in a wide variety of environmental conditions and at different times of the year.

3) In as much as possible, to establish the extent and ecological limits of the ecological range that aquatic bryophytes occupy, with particular consideration to their practical ubiquity.

4) To examine experimentally questions raised through field surveys regarding the factors affecting and the mechanism of metal uptake.

5) To integrate field and laboratory results in order to produce practical answers to fundamental and applied problems, with an evaluation of the statistical reliability of monitoring in general.

CHAPTER 2. METHODS

2.1 Introduction

2.11 Experimental design

Data were collected in a systematic manner in accordance with the computer-oriented records at Durham University for sampling of rivers for water, plants and sediments (Holmes & Whitton, 1977a, 1981; Whitton et al., 1979; Whitton & Diaz, 1980). This system permitted quick and efficient tabulation of data; in addition, comparisons could be made with present and past results at a particular site. Details of site designation are given in Section 2.2.

Collection of field data was part of a three-stage approach to studying heavy metal accumulation by bryophytes in streams and rivers. Field surveys, field experiments and laboratory experiments were integrated in order to test the results obtained from the previous step, the so-called "Hypethetico-Deductive" method (Fretwell, 1972). The method was applied to this study as follows: (1) collection of environmental data (observations) to describe conditions in nature; (2) formulation of hypotheses based on field results; (3) experiments run to test hypotheses; (4) compare experiments with field results.

2.12 Practical considerations

A part of the overall study was to evaluate methods used for applying aquatic bryophytes as monitors of heavy metal pollution (section 1.5). While new methods were developed in the course of the study they will be mentioned in the relevant methods sections, but the full details of these methods studies were not a direct part of this thesis.

2.13 Choice of organisms studied

Among a "package" of ten macrophytic plants recommended for monitoring metal pollution (Whitton et al., 1981b), five were bryophytes. In light of its considerable geographic and ecological ubiqity (sections 1.42, 1.43 pp. 41 - 54), Rhynchostegium riparioides was chosen for detailed study. Two other of these bryophytes, Scapania undulata (Whitton et al., 1982), and the moss Fontinalis antipyretica (Say & Whitton, in press) have already been the subject of study. The moss species Amblystegium riparium and F. antipyretica are also considered in this thesis, but in less detail.

2.2 Analytical methods

2.21 Routine laboratory procedures

Plastic equipment such as sample bottles, measuring cylinders and beakers were composed of high density polyethylene (often called "Polythene") or polypropylene which have been recommended for collection and storage of water samples (Batley & Gardner, 1977). Except for snap-cap vials, all glassware was composed of borosilicate glass ("Pyrex"). Vials, composed of soda glass, were tested for metal contamination of water and digest samples using blanks.

A system for the washing of plastic and glass containers was developed for the specific group of substances being analysed. Tap water was used (with detergent) for scrubbing very dirty equipment and containers. Two grades of distilled water were used for analytical purposes. Single distilled water was used in all cases for at least preliminary washing and for final rinsing of glassware in the analysis of some elements (e.g. phosphorus, pp. 69 -70) where blanks had indicated a lack of contamination. The second grade was a double distilled water which was then passed through an ion exchange column (Houseman model 3C deionizer). This latter grade will be referred to as "deionized water", although it was both double distilled and deionized.

Acid washing was carried out using three types of acids. Glassware and plastic containers used for analysis of metals, Cl^-, F^- and SO_4-S were soaked in 4% HNO_3 (Laxen & Harrison, 1981) for a minimum of 30 min, then rinsed six times in distilled water followed by three rinses of deionized water. Those used for nitrogen (NH_4-N, NO_2-N, NO_3-N) analysis were soaked for a similar time in 10% HCl and rinsed similarly. Glassware and plastic containers used for PO_4-P analysis were soaked in 10% H_2SO_4 for a minimum of 30 min, then rinsed ten times in distilled water. Polyethylene bottles used for collection of water for PO_4-P analysis were first iodine impregnated before acid washing (Mackereth <u>et al</u>., 1978).

2.22 Field methods

2.221 Field measurements in situ

At each site a number of measurements were made in situ: water temperature, pH, total alkalinity, dissolved oxygen and conductivity. Total alkalinity (TA) and pH were measured using an Orion (model 407A) pH/specific ion meter. The potentiometric titration for total alkalinity was carried out against 0.02N H_2SO_4 using a 1.00 or 5.0 ml syringe to endpoints pH 4.5 and 4.2 (American Public Health Association, 1981). Dissolved oxygen also employed the Orion meter, but was fitted with an oxygen probe (Orion model 97-08). Conductivity was measured using an Electronic Switchgear (model MCl mark V) conductivity meter corrected to 25 ^{o}C.

Other field measurements included first a visual estimate of percent cover of the streambed within the 10 m reach by <u>Rhynchostegium riparioides</u>. These were put into ranks of the nearest 10% and for low cover values assigned either 5-10%, 1-5% or < 1% cover. Second, a subjective estimate was made of the relative abundance of aquatic mosses within the reach, as compared with other macrophytes, based on the scale of Holmes and Whitton (1977a, 1981) for river macrophytes:

1 = rare	or	< 0.1%	
2 = occasional	or	0.1 - 1.0%	
3 = frequent	or	1.0 - 5.0%	of total macrophyte
4 = abundant	or	5.0 - 10.0%	biomass
5 = very abundant	or	above 10.0%	

The remainder of physicochemical and biological measurements were made from samples collected at each reach and brought back to the laboratory.

2.222 Collection of water

Water was collected in two 2 litre polypropylene beakers. Beakers were rinsed several times in stream water, then filled near to the brim. This water was allowed to stand for approximately 5 min to allow larger particles to settle out before separating the water into several fractions. Water from one beaker was filtered a through No. 2 size sintered glass ("Sinta"; Gallenkamp) funnels and collected in acid washed polyethylene bottles for anion analysis. Funnels were first rinsed with stream water from this beaker. Roughly the first 25 ml which filtered through each funnel was used to rinse the bottles and discarded. All were acid washed in accordance with the elements to be analysed (section 2.21: p. 65). Approximately 25 ml of water filtered by this procedure was also collected in an acid washed snap-cap vial for measurement of optical density.

Water from the second beaker was first poured unfiltered, into an acid-washed snap-cap vial (30 ml) and discarded, then filled near to the brim and capped. This sample is termed the "total" metal sample. A second "bulk total" sample was collected in a 100 ml Pyrex glass bottle for concentration in the laboratory. Third, a "filtrable" water sample for metals was collected by filtering water through a 0.2 µm poresize Nuclepore filter fitted in a Swinnex (Millipore Corp.) filter holder. Water was pushed through the filter using an acid washed borosilicate glass syringe. Some very turbid samples required two or three filters to collect enough water for a sample. This was also collected in a 30 ml snap-cap glass vial.

Water samples were stored in an ice box while in the field. In the laboratory the three anion samples from each site were frozen until analysis. The samples for metal analysis had two drops (snap-cap vials) or 5 drops (100 ml Pyrex bottles) of atomic absorption grade HNO_3 added to each. These were stored in a refrigerator (c. 4 ^{o}C) until analysis.

2.223 Collection of bryophytes

The sampling of aquatic bryophytes was restricted to plants which were fully submerged and as much as possible located within areas of maximum current velocity. Entire plants were collected from at least five separate localities or boulders within the reach. In the first ("intensive") survey, only Rhynchostegium was sampled for metal analysis. Mosses were then rinsed and shaken several times in stream water to remove associated sediment, invertebrates and any entangled filamentous algae. Excess water was then squeezed from the plants and samples were placed in an acid washed 500 ml polyethylene bottle. Mosses were also stored in an ice box in the field.

2.23 Laboratory methods for water

2.231 Analysis of anions in water

Frozen samples were thawed for 24 h immediately prior to analysis. There was concern that freezing may lead to precipitation of silica (Kobayashi, 1967), so samples were thoroughly mixed before analysis (Golterman et al., 1978). All colourimetric and turbidimetric determinations (three forms of nitrogen, silica, phosphate and sulphate) were measured on a Shimadzu (model UV-150-02) double beam spectrophotometer. The elements analysed are listed in order of atomic number.

Nitrogen

Three forms of nitrogen were analysed: ammonia (NH_4-N), nitrite (NO_2-N) and nitrate (NO_3-N) and analysis followed Mackereth et al. (1978) for all three. NH_4-N was reacted with phenol and sodium hypochlorite to form indophenol blue, measured at 635 nm. The detection limit during use was 5.0 µg l^{-1}. It was found necessary to add 0.5 ml of 0.2 M EDTA to a few high alkalinity samples to prevent precipitation with reagents. NO_2-N was reacted with sulphanilamide in an acid medium and subsequently coupled with NNED (N-1-napthylenethylenediamine dihydrochloride) which produced an intense red colour measured at 543 nm. The working detection limit was 1.0 µg l^{-1} NO_2-N. NO_3-N was analysed in the identical method as NO_2-N after reduction of NO_3 to NO_2 using spongy cadmium. Care was necessary to avoid severe phosphate interference of the reduction step (Davison & Woof, 1979; Olson, 1980) by dilution of samples whose PO_4-P concentrations were above 75 µg l^{-1}. The detection limit achieved for this method was 2.0 µg l^{-1} NO -N.

Fluoride

Fluoride was measured using an Orion specific ion F^- probe (model 94-09) using a total ionic strength buffer. The buffer consisted of 57 ml of acetic acid, 58 g NaCl and 4 g CDTA (cyclohexylene dintrilo-tetraacetic acid) made to one litre with deionized water. The pH of the buffer was adjusted to 5.3 with 5.0 N NaOH. CDTA was omitted from the buffer for levels below 0.4 mg l^{-1} F^-. The probe was fitted to an EIL (Electronic Instruments Ltd. model 7055) laboratory pH/specific ion meter. The minimum detectable concentration was 0.01 mg l^{-1}.

Soluble reactive silica

Silica was measured according to the ammonium molybdate method, described in Stainton _et al_. (1977), which produces a blue colour measured at 820 nm. The working detection limit obtained was 15 μg l^{-1} using a 1 cm cell.

Phosphate-P

Two forms of phosphate (filtrable) were measured: "filtrable reactive phosphate" (FRP) and "filtrable total phosphate" (= organic + orthophosphates), (Mackereth _et al_., 1978). Both involve the reaction of phosphate with molybdate in an acid solution. The organic forms were broken down with the addition of sulphuric acid and potassium persulphate before autoclaving, to analyse the "total" form. "Filtrable organic phosphate" (FOP) concentrations were then determined by the difference between the reactive and total forms. The detection limit achieved by these methods were 1.5 μg l^{-1} (10 cm cell) for filtrable reactive phosphate and 5.0 μg l^{-1} (4 cm cell) for filtrable organic phosphate.

It was found after completion of the study that the sulphuric acid - persulphate digestion also breaks down condensed inorganic phosphate (so-called "acid-hydrolyzable") which is not directly reactive with reagents used (American Public Health Association, 1981). This form apparently occurs in lesser amounts than others in most natural waters, but can enter water supplies from water treatment works. This may have caused overestimates of organic phosphorus in some rivers sampled.

Sulphate-S

Sulphate was measured using a turbidimetric method, which precipitates sulphate with barium chloride in an HCl medium and measured at 420 nm (American Public Health Association, 1981). The minimum detectable concentration was 0.4 mg l^{-1}.

Chloride

Chloride was measured using a Corning (model 003-59-091H) Cl^- specific ion probe fitted to a EIL laboratory pH/specific ion meter. No buffer was required and the detection limit was 1.0 mg l^{-1}.

2.232 Analysis of metals in water

"Total" and "filtrable" water samples were acidified as mentioned earlier, but otherwise were unchanged. "Total bulk" samples were concentrated (after acidification) from 100 or 200 ml to 10 ml by rotary evaporation (Slonim & Crawley, 1966). Samples were placed in a round bottom flask set in a 70 ^{o}C water bath. The flask was attached to a Buchi evaporator (type KR-65/45) and rotated while under vacuum. Tests of concentrated standards found acceptable precision between replicates (CV < 5%).

Metal concentrations were measured using a Perkin-Elmer (model 403) atomic absorption spectrophotometer. Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn and Pb were analysed in an air-acetylene flame, and Ba in a N_2O-acetylene flame. 10% $LaCl_2$ was added to samples (final concentration 0.68%) for analysis of Mg and Ca as a precaution against interference from silica, aluminium, phosphate and other elements (Perkin-Elmer Corp., 1973). For analysis of K and Ba, an addition of 15 000 mg l^{-1} NaCl was added to samples (10% v/v) to control ionization effects (Perkin-Elmer Corp., 1973).

Low level (< 0.05 mg l^{-1}) Pb and all Cd analyses of water samples were made using a flameless graphite furnace (Perkin-Elmer model HGA 74) in place of the flame burner. For both flame and furnace analysis of Cd and Pb, a background deuterium arc correction was employed. Due to changing properties of lamps and variations in signal/noise ratios, it was not possible to state an absolute detection limit for metal analysis. However, all water samples from a given survey were analysed at the same time so that a consistent detection limit can be stated for any set of results.

2.233 Other characteristics of water

Optical density (O.D.) of water samples was measured at wavelengths 240, 254 and 420 nm on the Shimadzu spectrophotometer. The samples used were filtered in the field with a sintered glass funnel to remove the majority of the suspended material.

2.24 Laboratory methods for bryophytes

2.241 Introduction

Considerable importance was placed on the standardization of methods for processing and analysing metals in aquatic bryophytes, so that comparisons of results from other workers could be made. During this study, some of these methods were being tested and improved (Wehr et al., in press), which resulted in some changes being employed later in the study, explained below.

2.242 Storage, fractionation and washing

Mosses collected from the field were stored in acid washed polyethylene bottles in the refrigerator (4 ^{o}C) for no more than two days before further treatment. For washing, mosses were placed in an acid washed nylon sieve and first rinsed under running distilled water to remove any further sediment, algal filaments or unwanted bryophyte species. Material was then transferred to a series of crystallization dishes filled with deionized water (the first was one litre in capacity). From this, selected shoots or plants were removed using stainless steel forceps and scalpel to a petri dish containing deionized water. Plants were measured and apical 2 cm tips of the shoots were removed, which were transferred to a smaller (100 ml capacity) crystallization dish for a final rinse in deionized water.

The amount of material used depended on the survey or experiment, but a minimum of about 30 tips was usually sufficient to produce a dry weight of between 50 and 100 mg. In the intensive survey, between 100 and 300 mg dry weight was used to improve detection of trace amounts of elements in the digests. A separate study (in preparation) found that increasing dry weights of bryophyte material used for digestion had a negligible effect on the results obtained. For the intensive survey (section 2.33: p. 75) whole plants of Rhynchostegium were processed and washed in an identical manner to the 2 cm apical tips.

2.243 Drying and Weighing

Apical tips or whole moss samples were dried in an oven at 105 ^{o}C for a minimum 48 h but no more than 72 h. Prior to weighing, material was cooled in a desiccator over silica gel. Samples which exceeded 100 mg were ground to a fine powder using an acid washed mortar and pestle. These samples were then re-dried at 105 ^{o}C and cooled in a desiccator. A comparison between ground and unground moss samples found no significant differences ($p < 0.05$) in metal concentrations.

Samples were placed on a dried filter paper and weighed by difference using a Metler (model H16) analytical balance to the nearest 0.05 mg. A beaker of dried silica gel was placed in the weighing chamber to reduce atmospheric moisture during weighing.

2.244 Digestion and analysis

In the course of the study, tests were made on the efficiency of several acid types which pointed out the need to change methods. Results showed a small (up to 5%) but significant improvement in accuracy was obtained by changing the molarity of the acid used (section 1.52: p. 61). To assure consistency in results, one group of studies maintained the use of the earlier procedure; the remainder (and the majority) of samples were digested using the lower molarity.

After weighing, samples were placed in Pyrex boiling tubes (20 ml capacity). To this 5 ml of atomic absorption grade nitric acid was added. The tubes were heated to 150 ^{o}C in a Tecam Dri-Block (model DB-3H) heating rack for 30 - 45 min, or until boiling had ended. Some samples (transplant experiments) were digested using concentrated nitric acid, while all later samples were digested using 2 M. After digestion the samples were cooled, centrifuged to remove sediment or undigested material and made up to a 25 ml volume with deionized water. The final digest was then transferred to an acid washed snap-cap vial. Blanks of acid only were processed in an identical manner.

Digests were analysed for metals by atomic absorption spectrophotometry. It was found that the presence of nitric acid in the digests inhibited measurement of calcium and nickel, so all standards were made up in equivalent molarity nitric acid.

2.3 Field surveys

2.31 Introduction

Because rivers are subject to considerable temporal change, single samples from one point in time may not be entirely representative of a given section of river. Therefore, the strategy adopted for field surveys was as follows: 1) to sample from a reasonably large and diverse array of sites within a short period of time; and 2) to sample from a few selected sites at regular intervals over a year to examine temporal and/or seasonal effects.

2.32 Reconnaissance surveys

It was decided that preliminary visits to streams and rivers throughout northern England was necessary to assess the distribution of *Rhynchostegium* in the region and to assemble information on metal concentrations of river water and mosses. Many of these trips were either in conjunction with field assistance for other workers (e.g. Burrows, 1981; Say *et al.*, 1981; Patterson & Whitton, 1981) or follow-up visits to streams where other studies had been conducted (e.g. Say, 1977; Holmes & Whitton, 1977a, b, c; Say & Whitton, 1977;

Harding, 1978). Exploratory surveys of streams not previously sampled by workers in Durham also uncovered many useful sites.

During these visits a reduced "package" of measurements and samples included pH, "total" metals and a bryophyte sample. From these collections, records from the literature and several personal communications, it was possible to assemble a list of over 100 locations which were suitable for an efficient collection of a large amount of material in a reasonably short span of time.

2.33 Intensive survey of 105 river sites

A total of 105 sites were sampled in a six week period between May and June 1981. The sampling area at each site was a 10 m length of stream or river which was decided to be typical of the complete section being considered. This 10 m length is termed a "reach" (Holmes & Whitton, 1981). The location of each reach was defined within the Durham recording system by a grid reference number using 1:25 000 or 1:50 000 Ordnance Survey maps and given a four digit stream number and a two digit reach number. Samples were collected when the streams were in average flow. In as much as possible, the reaches were relatively homogeneous chemically and as such no tributaries or effluents entered the stream within a reach. There was one sampling site, a sewage effluent (0360-01), which was less than 10 m in length. All in situ measurements and samples outlined in section 2.22 (pp. 65-67) were in-included in this survey and only Rhynchostegium riparioides was collected for analysis.

2.34 Seasonal survey of seven rivers

Objectives for this survey were somewhat different from the intensive survey and involved a slightly different approach. The seasonal survey included periods when extremes of flow and other physical factors were met. Sampling sites were studied for one year, usually at monthly intervals between May 1981 and June 1982. The environmental background for these sites is given in section 3.3. Sampling was carried out each month in what was regarded as a "typical" flow period for that month, based on regular observations of the River Wear in Durham. Weather conditions prevented sampling on every month at all sites. The River Wear was frozen from December through January and sampled 10 out of 14 months. The River Team was sampled 13 times and all others were sampled on 11 months out of 14.

Procedures concerning the use of a 10 m reach and sampling discussed earlier were also followed in seasonal surveys. The same sampling strategy was followed as in the intensive survey, with a few exceptions: two water samples were collected for anion analysis instead of three; no "bulk" water samples were collected for concentration; only apical tips of bryophytes were used for metal analysis. Three species of aquatic bryophyte were collected in these surveys: Amblystegium riparium (River Team at Causey Arch, 0024-20), Fontinalis antipyretica (River Wear, 0008-65) and Rhynchostegium riparioides (all seven sites).

2.4 Field experiments

2.41 Measurements and sampling

Rhynchostegium riparioides was used exclusively for transplants. Boulders with mosses attached were collected from stream(s) and carefully placed in plastic buckets containing streamwater from the site for transport. Where more than one population was used, boulders were labelled with bright yellow paint for identification. To avoid decimating a population from an established sampling reach, boulders were usually collected just upstream or downstream from the exact 10 m reach.

Boulders were transplanted into a site with either increased or decreased aqueous metal concentrations and sampled at timed intervals, usually over 24 or 48 h. Rhynchostegium also was present *in situ* at all experimental sites. The experimental design depended on the specific question(s) being asked (see below).

A routine series of measurements and procedures were carried out. At each sampling interval "total" and "filtrable" water samples were collected for metal analysis and temperature and pH were measured. Moss samples were rinsed and fractionated in the field during the intensive sampling from 0 - 12 h. For all experiments, 2 cm apical tips (section 2.242) were used. After fractionation, moss tips were placed into acid washed snap-cap vials and kept in an ice box. These vials were also used later for drying the mosses as well and storing the digested material. During some time intervals (usually 0, 4 and 12 h) five replicates of mosses were collected to estimate variability during the experimental period.

2.411 Cross-transplants

In the first experiment mosses were cross-transplanted between two sites on the River Team (0024-05 and 0024-20), which were above and below the entrance of a zinc effluent (further details of sites are given in sections 3.2 - 3.3). One worker was stationed at 0024-05 in a tent over the first 20 h and at the other site work was carried out from a car. This experiment was designed to compare rates of zinc uptake and loss.

2.412 Nutrient effects

Mosses were collected from a comparatively unpolluted upper reach on the River Browney (0014-14) and transported to two streams with similar aqueous zinc concentrations but markedly different "nutrient" regimes: Rookhope Burn (0012-45), an upland "low nutrient" site and the River Team (0024-20), a lowland "high nutrient" site. Previous surveys had shown the latter site to have significantly greater concentrations of nitrate and reactive phosphate. This experiment was designed to compare zinc uptake in these two regimes.

2.413 Interpopulation differences - I

Populations from five different "low zinc" (< 0.05 mg l^{-1}) sites were collected: "Race Fell Burn" (0310-90), Thornhope Beck (0284-85), River Browney (0014-14), Chester Sike (0355-95) and Stoney Gill (0356-85). These were first transported to a common "low zinc" stream, Waskerly Beck (0123-50), for two weeks to equalize any differences in metal concentrations in the mosses. Boulders were then transplanted to an upland, mining polluted stream, the River Nent (0048-90), to compare rates of accumulation. Mosses were placed in similar riffles within the reach (current velocity was compared using an Ott (model 10.150 C) current meter). An experiment was conducted simultaneously in this stream to examine the effects of current velocity (section 2.415: p. 79).

2.414 Interpopulation differences - II

A comparison was made of the accumulation of metals by two populations which had been growing in markedly different heavy metal concentrations (e.g. 1.0 v. 0.03 mg l^{-1} Zn). Although no direct experiments conducted, they were assumed to differ in their tolerance to heavy metals, as a zinc-tolerant population of Hormidium rivulare had been isolated from the contaminated stream (Say & Whitton, 1977). Boulders with the "tolerant" population ("High Crag Burn," 0101-05) were moved to a site with lower heavy metals ("Race Fell Burn," 0310-90) and allowed to equilibrate with the *in situ* population for 15 days. Both populations then were transplanted into "High Crag Burn" to compare metal accumulation.

2.415 Current velocity effects

Mosses from "Race Fell Burn" were placed in a pool with negligible current velocity (< 2 cm s^{-1}) and in a riffle with a rapid current velocity ($\bar{x}$ = 145 cm s^{-1}) in the River Nent. This was run concurrently with the first interpopulation study.

2.5 Laboratory accumulation experiments

2.51 Introduction

Many questions were raised through field surveys and transplant experiments which were the result of a combination of factors which differed between sites and over time. Some variables were often interrelated (e.g. Ca, Mg) and their effects could not be separated. Although conditions in the laboratory could not entirely simulate conditions in a stream, a standard, repeatable regime was created which was designed to isolate individual effects. Thus, several experiments were specifically designed to follow up field results.

2.52 Experimental material

Bryophytes were collected from one reach, "Race Fell Burn"

(0310-90), where a large biomass of material was present year-round, plants were reasonably free of epiphytes and streamwater had relatively low levels of heavy metals (e.g. < 0.05 mg l^{-1} Zn). For most experiments, Rhynchostegium riparioides was used, although Fontinalis antipyretica was compared in one study.

2.53 Culture techniques

2.531 Preparation of moss for assay

Material collected from the field was used for experiments within three weeks of collection; plants left unused in the laboratory after this time were discarded. Mosses were washed and fractionated into 2 cm apical tips and stored in polyethylene bottles. Material was found to keep well in the refrigerator until use.

Prior to the experiments, a test was made comparing tips which were rinsed with approx. 4% hypochlorite (bleach) to surface sterilize the moss before incubation (Hamilton, 1973), with those only rinsed with several changes of deionized water. Tips were placed into a 47 mm Swinnex filter holder and hypochlorite solution was sprayed through the tips for about 10 seconds. Tips were then rinsed in several changes of sterile deionized water. No obvious difference was found between surface sterilized and normally rinsed tips when kept in culture for 10 days and no differences in algal contamination were noted between the two treatments. As uptake experiments were usually short term (24 or 48 h), it was decided that this pretreatment was unnecessary.

2.532 Culture medium

The medium used was based on the No. 10 recipe of Chu (1942); a formula at Durham University termed "Chu 10E", with several modifications (Table 2.01). This included the "C" microelements stock of Kratz and Meyers (1955), but with no added zinc and reduced manganese. Without added zinc, concentrations of zinc in the medium were < 0.004 mg l^{-1}. The pH was buffered using 2.5 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid: Good et al., 1966), and the pH adjusted to 7.5 (± 0.05) with 2N NaOH. This buffer was found to have negligible chelation effects on zinc (G. Patterson, pers. comm.).

Zinc was added to the medium either as $ZnSO_4.7H_2O$ or in later experiments this was used as a carrier with ^{65}Zn added as a tracer in the form of $^{65}ZnCl_2$ in a 0.2N HCl solution (obtained from Amersham International, U.K.). The main ^{65}Zn stock had an initial total specific activity of 2.0 mCi when obtained, or 0.84 mCi ml^{-1}; which corresponded to 1.03 mg ml^{-1} Zn. Working stocks were made by diluting 24 µl to 5.0 ml to produce a concentration of 5.0 mg l^{-1} ^{65}Zn. For each litre of medium prepared, 2.5 ml of working stock was added, giving a final concentration of 0.0125 mg l^{-1} ^{65}Zn as a tracer. This represented 1.2% of the total zinc in the medium for most experiments. The medium was altered to test effects of various elements on zinc uptake. Stock solutions of each element were made up for additions or replacements.

Calcium

A medium free of calcium was prepared by replacing $Ca(NO_3)_2$ with an equimolar nitrate solution of $NaNO_3$. Calcium was added to the medium from 2000 and 20000 mg l^{-1} $CaCl_2.2H_2O$ stocks. A range of 2.5 - 110 mg l^{-1} was based on the range of concentrations measured in the field for Rhynchostegium sites (same for other elements tested). The normal level of calcium in Chu 10E was 9.78 mg l^{-1}.

Table 2.01 Concentrations (mg l^{-1}) of salts in modified "Chu 10E" medium used for accumulation experiments, prior to the addition of zinc (* = added together in one solution).

component	salt	concentration
basal medium		
	$Ca(NO_3)_2.4H_2O$	57.6
	KH_2PO_4	3.9
	$MgSO_4.7H_2O$	25.0
	$Na_2SiO_3.5H_2O$	10.9
	$NaHCO_3$	7.9
Fe EDTA*		
	$FeCl_3.6H_2O$	0.24
	$Na_2EDTA.2H_2O$	0.32
	Microelements*	
	$MnCl_2.4H_2O$	0.012
	$NaMoO_4.2H_2O$	0.007
	$CuSO_4.5H_2O$	0.019
	$CoSO_4.7H_2O$	0.010
	H_3BO_3	0.73

Magnesium

A magnesium-free medium was prepared by replacing $MgSO_4.7H_2O$ with an equimolar sulfate solution made from $NaSO_4$. Magnesium was added from a 5000 mg l^{-1} stock made from $MgCl_2.6H_2O$. The range of concentrations tested were 0.5 - 80 mg l^{-1}. The normal level of magnesium was 2.47 mg l^{-1}.

Phosphate-P

Phosphorus additions were provided from the original Chu 10E K_2HPO_4 stock and ranged from 0.10 - 10 mg l^{-1} PO_4-P in the medium. The normal level of PO_4-P was 1.16 mg l^{-1}.

Nitrate-N

A medium free of nitrate was prepared by adding equivalent amounts of calcium from a 2000 mg l^{-1} $CaCl_2$ stock instead of $Ca(NO_3)_2$. Nitrate was added to the medium separately from an equimolar NO_3-N stock made from $NaNO_3$. The range of concentrations tested were 0.025 - 10.0 mg l^{-1} NO_3-N. The normal concentration of nitrate-N was 6.8 mg l^{-1}.

Manganese

Manganese was added to the medium from 100 ppm Mn solution prepared from $MnCl_2.4H_2O$. No attempt was made in this experiment to prepare a Mn-free medium. However, "trace" levels from the microelement stock resulted in a minimum concentration of 0.012 mg l^{-1}. The range of concentrations tested ranged from 0.012 - 5.0 mg l^{-1}.

Silica

Silica was added to the medium from the Chu 10 $Na_2SiO_3.5H_2O$ stock.

Concentrations of added Si to the medium tested ranged from 0 - 15.0 mg l^{-1}. The normal silica concentration of the medium was 1.62 mg l^{-1}. As some workers have suggested silica may act on zinc availability through binding (Hem, 1972), the complete medium was prepared and allowed to react and/or reach equilibrium for 24 h before beginning the experiment.

Humic acids

Humic acids stocks (HA) were prepared from a commercially prepared reagent (Aldrich Chemical Co., Ltd.; lot no. Hl, 675-2). The HA was purified according to methods modified from Weber and Wilson (1975) and Schnitzer and Kerndorff (1981):

1) 2.0g HA dissolved in 0.01N NaOH and made to 1 litre and allowed to stand for 48 h.
2) The alkaline extract was acidified to pH 2.0 with concentrated HCl and allowed to stand for 48 h.
3) After precipitation and settling, some of the supernatant (primarily fulvic acids), was carefully pipetted off.
4) The coagulate was washed with several changes of 0.05N HCl and centrifuged at 3200 rpm for 10 min after each wash.
5) Coagulated HA was rinsed with a minimum of deionized water into acid washed petri dishes and dried slowly at 50 °C.
6) Exactly 1.0000 g of dried HA was weighed out and redissolved in 0.01N NaOH to produce a 1000 ppm stock, which was stored in a refrigerator.

Concentrations of 0 - 40 mg l^{-1} HA were tested against zinc accumulation. The media were also allowed to equilibrate with HA for 24 h before beginning the experiment. A calibration curve of optical density at two ultraviolet wavelengths against concentration was run to compare with field measurements of optical density.

2.533 Culture apparatus and experimental regime

For all experiments 100 ml of media and 5 or 10 bryophyte tips (depending on the experiment) were added to 250 ml "Pyrex" glass Ehrlenmeyer flasks. Flasks were closed using cotton-wool bungs for normal zinc experiments and silicon sponge rubber bungs (Sanko Plastics, Japan) for flasks with ^{65}Zn. The latter could be washed if contaminated. Flasks were placed in a water bath shake-tank. The entire experiment was maintained in a 15 ^{o}C "cool room". The shaker produced a 3.5 cm horizontal oscillation at a rate of approx. 90 shakes per minute. Temperature was maintained at 15 ^{o}C ($\pm$1 ^{o}C) using a mercury thermometer-based thermostat heating/cooling unit. Continuous subsurface illumination was provided by a bank of "Day-Light" fluorescent lights which produced an irradiance of approx. 100 $\mu E\ m^{-2}s^{-1}$ within the portion of the tank used. Care was taken to place all flasks in the central area of the tank, as tests showed a marked drop-off in irradiance near the edges.

2.54 Measurement of zinc accumulation

Initial experiments with only normal zinc in the medium used five separate flasks, each with 5 or 10 apical (2 cm) tips of moss in each. At the end of an incubation period, tips plus media were poured into an acid-washed crystallization dish. Tips were rinsed in four changes of deionized water, blotted on absorbent paper and placed in glass snap-cap vials to be dried at 105 ^{o}C. Tips were digested and their zinc concentrations measured by atomic absorption spectrophotomety.

In experiments using ^{65}Zn as a tracer, five tips were incubated in each flask. Because of the high sensitivity of ^{65}Zn measurement, individual tips were used as replicates, usually with one flask per treatment. A preliminary experiment (section 7.321) compared variability within these five replicates with five separate flasks and found no significant difference ($p < 0.05$) in concentrations. At the end of an incubation period, the media were carefully poured into a 2 litre Ehrlenmeyer flask for later disposal. The culture flask plus moss tips were rinsed twice with distilled water, the rinse was also poured into a waste flask and the tips were poured into a crystallization dish. Tips were rinsed further in three dishes of deionized water and then blotted dry on absorbent paper. Routine tests with a portable gamma probe (Nuclear Enterprises, Ltd.; model GP7) found that no surplus ^{65}Zn was detectable in any of the last three rinsing dishes or on the blotting paper. The probe was also used for general checks for contamination of glassware and lab equipment. Rubber gloves were worn for handling radioisotopes and all equipment in these experiments.

For counting, each tip was placed in a separate plastic scintillation vial which was held in groups of ten in a vertical counting rack. Activity of ^{65}Zn in the tips was measured using a Beckman Gamma 4000 Counting System. Counts from moss samples were compared against a standard slope of known ^{65}Zn standards which were recalibrated every experiment to account for natural decay of the isotope. Five blank vials were measured every run for background correction. Tips were dried and weighed as described in section 2.243 and calculations were made in four steps:

1) mean of standards: $(\text{counts min}^{-1})/(\mu g\ ^{65}Zn) = \text{slope}$

2) for sample: $(\text{counts min}^{-1})/(\text{slope}) = \mu g\ ^{65}Zn$ in sample

3) $(\mu g\ ^{65}Zn \text{ in sample})/(\text{dry wt.: (g)}) = \mu g\ ^{65}Zn\ g^{-1}$ of sample

4) $$(\mu g\ g^{-1}\ ^{65}Zn)\ \frac{\text{total Zn in medium}}{^{65}Zn \text{ in medium}} = \mu g \text{ total Zn } g^{-1} \text{ of sample}$$

After completion of an experiment all radioactive media plus tips were disposed of (with date recorded) in the Botany Department radioisotope wet waste disposal unit.

2.6 Localization

2.61 Fractionation studies

Rhynchostegium was collected from several field sites to examine differences in concentrations of metals in different portions of the plant. Plants were washed and processed in an identical manner as described previously, except for the method of fractionation. In the first group of studies mosses were measured and cut into successive 1 cm fractions from the apical tip to the base. The second study compared differences between metals in leaves and stems of the moss by stripping off leaves with two pair of stainless steel forceps. For both procedures the material was dried, digested and analysed by atomic absorption as described previously.

2.62 Experimental study

Comparisons were made of the accumulation of ^{65}Zn by successive cm sections of moss. Instead of five 1 cm sections, each flask contained a single section which was 5 cm long. These were incubated as described previously (Section 2.4), using identical radiotracer methods as in accumulation experiments and two exposure periods (4 h, 24 h). At the end of each period, the moss was fractionated into five 1 cm sections down the stem and measured for ^{65}Zn content.

2.7 Taxonomy

2.71 Floras used for aquatic bryophytes

The bryophyte floras which were consulted include Wilson (1855); Braithwaite (1887-1905); Dixon (1924); MacVicar (1926); Arnell (1954); Nyholm (1954-1969); Watson (1968); Smith (1978) and Crum & Anderson (1981). The only flora which deals strictly with aquatic bryophytes is from Pascher's Süsswasserflora series by Warnstorf _et al._ (1914). Because nomenclature between authors differed and had changed over time, the British authority (Smith, 1978) was recognized for mosses and the recommendations of Holmes _et al._ (1978) were followed for names of aquatic liverworts.

2.72 _Rhynchostegium riparioides_ (Hedw.) C. Jens.

There were specific problems regarding the identification and nomenclature of _R. riparioides_. Many aquatic bryophytes exhibit considerable variability in growth form within a species (Nyholm, 1954-1969; Watson, 1968; Smith, 1978; Crum & Anderson, 1981), which is particularly true for _Rhynchostegium_. Early in the study it was observed that in several rivers with organic pollution this species developed a morphology similar to _Amblystegium riparium_. This has also been observed in rivers in Belgium (A. Empain, pers. comm.). It can also be confused at times with aquatic _Hygrohypnum_ and _Brachythecium_ species (Smith, 1978; W. B. Schofield, pers. comm.). Warnstorf _et al._ (1914) have stated that " . . . it is a thankless task . . ." to unravel the numerous forms and varieties of this species which have been described.

Due to the extreme variability of this species, none of the subspecific taxa which have been reported were recognized. Based on field observations and the taxonomic works considered, the following criteria were found to be most useful in distinguishing R. riparioides from other taxa in the Amblystegiaceae and Brachytheciaceae:

1. Plants in the field fairly to highly robust, often with coarse texture.
2. Lower stems usually stripped of leaves, often blackish below.
3. Leaf shape typically broadly ovate.
4. Leaves at least minutely or often strongly denticulate along the margins from the base and extending at least 3/4 up the leaf.
5. Leaves with a distinct, single and undivided costa; usually extending about 2/3 up from the base.
6. Sporophyte, when present (common autumn - winter), charcterized by a capsule with an operculum (lid) having a well developed beak.

Species of Hygrohypnum and Amblystegium were never observed to exhibit all of these characteristics, nor did all specimens referred to R. riparioides. Thus, the full impression of all these characters was necessary for identification. Specifically, H. luridum and H. ochraceum can be distinguished by the lack of characters 1, 2, 5 and 6, as well as posession of auriculate leaf bases. A. riparium was separated from R. riparioides in usually lacking characters 1, 3, 4 and 6.

Nomenclatural confusion regarding the status of R. riparioides is considerable and has been discussed elsewhere (Lewis, 1974; Smith, 1977, 1978; Crum & Anderson, 1981). However, several synonyms were recognized which were important in locating other relevant ecological works in the literature:

Hypnum rusciforme Brid.
H. riparioides Hedw.

Hygrohypnum nicholsii Grout

Eurhynchium rusciforme (Br. Eur.) Milde
E. rusciforme (Neck.) Milde
E. riparioides (Hedw.) Rich.

Platyhypnidium rusciforme (Br. Eur.) Fleisch.
P. riparioides (Hedw.) Dix.
P. riparioides Podp.

Oxyrhynchium rusciforme (Neck.) Warnst.
O. riparioides (Neck.) Jenn.

Rhynchostegium rusciforme Br. Eur.
R. rusciforme v. inundatum Kindb.
R. rusciforme v. inundatum (Brid.) BSG
R. riparioides (Hedw.) C. Jens.

In this study the binomial R. riparioides was adopted as this was used by the most recent British authority, Smith (1978). However, a review of Smith's flora (Anderson, 1980) specifically disagreed with this treatment and Crum and Anderson's (1981) later North American Flora continues to recognize E. riparioides.

2.8 Morphometric analysis

2.81 Morphological characters

In light of the complex morphological variation in Rhynchostegium, herbarium speciments of this moss from the 105 survey sites (section 2.33) were examined for a series of key features. Gametophytic characters were used exclusively for analysis as collections were made in May and June when sporophytes were absent and because many characteristics of the sporophyte (e.g. seta colour and texture, capsule and operculum shape) were quite consistent among populations reference to the major taxonomic references, 15 gametophytic characters termed "operational taxonomic units" (Sneath & Sokal, 1973) were scored for each population (Table 2.02).

2.9 Computations and statistics

2.91 Facilities and software

Computing facilities were provided through the Northumbrian Universities Multiple-Access Computers (NUMAC) using an IBM 360/370 mainframe located at Newcastle University. The system operated under the Michigan Terminal System (MTS) which also provided most of the programs used for data analysis.

Many of the statistical and computational analyses were performed with aid of programs in the MIDAS statistical package (Fox & Guire, 1976). The MTS public file program *DIABOLIC was used for producing text for this thesis.

2.92 Organization of datafiles

Field and laboratory data were organized into files in x/y arrays where "variables," i.e. different categories of measurable elements (e.g. pH or weight) were arranged in columns and "cases," i.e. separate measurements of a variable (e.g. dates or sites) in rows. At the simplest level this permitted calculations of descriptive statistics of all variables (mean, variance, standard deviation, minimum, maximum), as well as computations such as calculating metal concentrations in plants from atomic absorption values and dry weights, directly by computer.

Table 2.02. Operational taxonomic units scored for 105 populations of Rhynchostegium riparioides.

character	scale or units
plants	
1. maximum primary axis length	cm
2. maximum leafy axis length	cm
3. maximum (unbranched) primary node length	cm
4. $\bar{x}$ apical tip weight	mg
5. "robustness"	(1) flaccid to (5) robust
6. colour	(1) light - bright green; (2) medium - dark green; (3) yellow-brown - black
7. proportion leafy	(1) <1/3; (2) 1/3-1/2; (3) >1/2
8. branching	(1) parallel; (2) intermediate; (3) dendroid
leaves	
9. length	mm
10. mid-leaf width	mm
11. length/width ratio	unitless
12. shape	(1) ovate; (2) elliptical; (3) lanceolate
13. margin denticulation	(1) < 10%; (2) 10 - 33%; (3) < 33 - 66%; (4) > 66%
14. nerve length (proportion)	(1) < 1/2; (2) 1/2 - 3/4; (3) >3/4
15. angular cell shape	(1) rectancular; (2) rhombic; (3) oval

2.93 Normalization and standardization of variables

Many statistical procedures (esp. parametric analyses) required certain assumptions to be satisfied or at least examined prior to beginning analysis. The variables were tested to determine whether the error variation is (1) homogeneous; (2) normally distributed; and (3) independent of the mean (Elliot, 1977; Green, 1979). These were first tested by creating histograms of all variables after standardizing them according to the formula:

$$Zi = (xi - \bar{x}) / S^2$$

where Zi= standardized variable for case i, xi= measurement of x for case i, $\bar{x}$ = mean of all cases, and S^2 = the variance. Thus, normality of a given variable was considered in two ways. First by the shape of the distribution curve of the histogram, that it was centered and concentrated around the mean. Second, the $\pm$ 1 standard deviation (SD) about the mean should correspond to 68% of all observations and $\pm$ 2 SD to 95% (Statistical Research Laboratory, Univ. Michigan, 1976). The second set of tests were calculations of skewness (g1: "symmetry") and kurtosis (g2: "peakedness") coefficients (Snedecor & Cochrane, 1967). Those which did not satisfy these assumptions were either appropriately transformed (e.g. $\log_{10}$) or nonparametric methods were used. Parametric tests which were used also included a test of the independence of the variance from the mean.

Some water chemistry data included measurements below the detection limit used and were recorded as less than this level, rather than "zero." These values could not be used for statistical analyses in this form. The procedure used in these circumstances was to halve the detection limit value (Diaz, 1980). This prevented any loss of data and was a better estimate than either "zero" or the detection limit value. Only those variables which had 10% or less of the total below this level were treated in this manner because of biases introduced by such a compromise. Those with a greater percentage below the detection limit were not included in statistical analyses.

2.94 Nonparametric methods

Two groups of nonparametric statistical procedures were employed for data analysis: (1) multivariate analyses, principally used for summarizing data sets; and (2) correlation analyses, which were used for comparing linear relations between pairs of environmental variables. Multivariate methods used include an ordination of sites (using principal components analysis) to summarize results of field surveys. Simple linear regression analyses were used to interpret what significant relations exist between environmental variables, as well as a test of the linearity of uptake rates. A multiple stepwise regression (Mead, 1971) was used to consider what factors may have influenced metal accumulation in field populations of mosses. A time-series analysis of seasonal data incorporated a cross-correlation analysis, using three successive lags of accumulation.

2.96 Parametric tests

Hypothesis testing using parametric methods was carried out largely via analysis of variance (ANOVA) or Student's t-test. For both, the null hypothesis (Ho) was that the two population means were equal (ul = u2). Ho was rejected if t or f values were great enough to indicate the probability of this occurring due to chance was less than 5% ($p < 0.05$).

CHAPTER 3. STUDY AREAS

3.1 Introduction

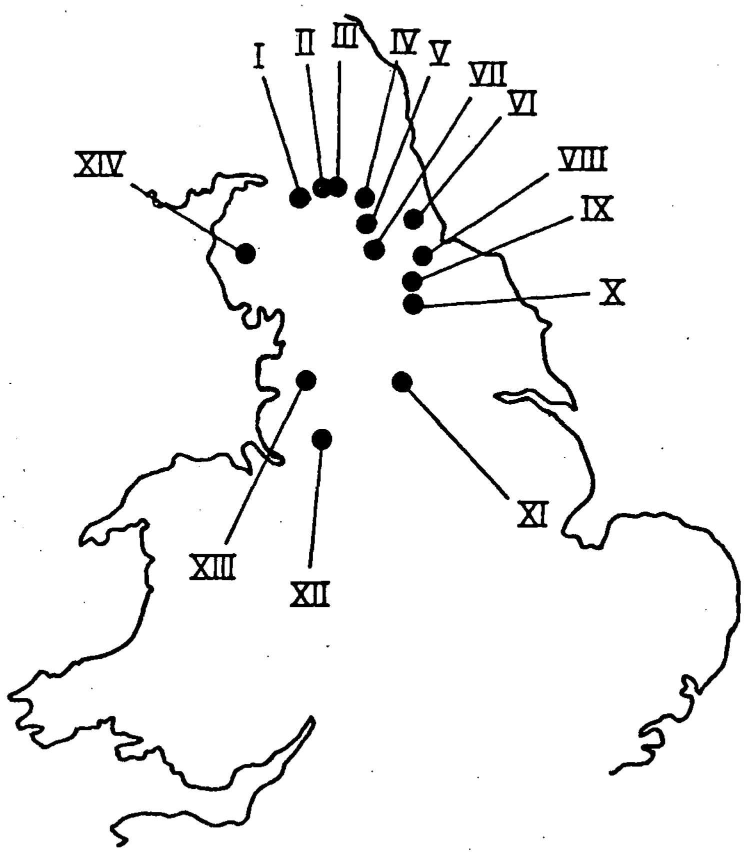
This chapter introduces the areas where field surveys and field experiments were carried out. Collections were made throughout northern England, and primarily in the Northern Pennines. Sites were chosen to cover as wide a range of physical and chemical characteristics as possible where Rhynchostegium riparioides could be found.

The geographic and physical aspects are first discussed, followed by some background information on geological and industrial sources of heavy metal contamination in the area overall. The study area is divided into 14 hydrogeological regions primarily based on the classifications of Eastwood (1921), Bromehead et al. (1933), Dunham (1948, 1974) and Earp et al. (1961). Because such a wide area has been covered, descriptions of the geology of each of the 14 regions are not given in great detail. Descriptions of the seven stream sites which were studied monthly follow (section 3.3: p.108), with general comments on the geomorphology, mining or other pollution and vegetation within their immediate area.

3.2 Geographical location and physical characteristics

The geographic location of the 14 sampling regions in northern England are shown in Fig. 3.01. Details, including the names, grid references and sampling dates are given below.

Figure 3.01. Map of location of hydrogeological sampling regions.



I	Alston Moor:	10 sites	VIII	Lower Tees:	2 sites
II	West Allendale:	5 sites	IX	Arkengarthdale:	12 sites
III	East Allendale:	4 sites	X	Swaledale:	13 sites
IV	Derwent Valley:	4 sites	XI	Holme Valley:	3 sites
V	Weardale:	17 sites	XII	Mersey Catchment:	8 sites
VI	Durham Coalfield:	9 sites	XIII	Ribble Catchment:	3 sites
VII	Teesdale:	8 sites	XIV	Lake District:	7 sites

3.21 Region I: Alston Moor

The drainage areas of the Rivers South Tyne and Nent are perhaps the most rugged landscapes of all the regions sampled. Altitudes range from about 300 - 700 m in open moorland and agricultural grazing land. The major town of the area is Alston and the other principal village is Nenthead, where much of the mining activity was centred in the past. Geological features in this region (as well as regions II, III), are dominated by the presence of the Great Limestone (Dunham, 1948; Johnson, 1981) which occurs in a large band to depths exceeding 20 m. Within the limestone are located the veins bearing heavy metals. The 10 sampling sites (Table 3.01) are associated with several disused lead, zinc, barytes and fluorspar mines. Copper and iron ores were also worked to a lesser degree. Many mines, which bear the same names as the streams, have been in operation for several centuries, but activity was most intense in the 18th and 19th centuries (Dunham, 1948). Although the majority are disused, there is a continued release of metals to surface waters from old tailings, hushes and adits (Dunham, 1981). Amongst limestone boulders and cobbles in the rivers, particularly the R. Nent, galena and fluorspar can be found.

Table 3.01. Sampling sites in Alston Moor.

name	stream-reach	grid ref.	date sampled
High Crag Burn	0101 - 05	NY 737424	06/05/81
Garrigill Burn	0102 - 85	NY 743419	06/05/81
River South Tyne	0055 - 30	NY 716467	06/05/81
" " "	0055 - 31	NY 716469	06/05/81
Ash Gill	0287 - 85	NY 758405	06/05/81
Nattrass Gill	0285 - 65	NY 727448	06/05/81
River Nent	0048 - 90	NY 723467	06/05/81
" "	0048 - 98	NY 716467	06/05/81
Blagill Burn	0288 - 98	NY 739470	06/05/81
Hudgill Burn	0167 - 50	NY 753459	03/06/81

3.22 Region II: West Allendale

The River West Allen and its tributaries lie within an area which begins as open moorland near its head and continues through well-established estates, principally the grounds of Whitfield Hall. Geological features similar to Alson Moor with several mines in the area being extensions of veins worked in Nenthead. Although Dunham (1948) records far less mining activity and production within this region as compared with East Allendale, the river system has shown greater problems of heavy metal pollution (Say, 1977; Abel & Green, 1981). Five sampling sites in the region (Table 3.02) lie within altitudes of 200 - 300m.

Table 3.02. Sampling sites in West Allendale.

name	stream-reach	grid ref.	date sampled
Lee Springs	0289 - 98	NY 781518	14/05/81
Mohope Burn	0312 - 90	NY 781518	14/05/81
" "	0312 - 95	NY 781519	14/05/81
River West Allen	0085 - 50	NY 782523	14/05/81
" " "	0085 - 85	NY 782571	14/05/81

3.23 Region III: East Allendale

Running parallel to region II is East Allendale. This dale originates near the town of Allenheads, the location of the most productive lead mine in the orefield (Dunham, 1948). A long history of mining has continued until recently with reclamation of the Allenheads mine by British Steel Corporation (now disused). Great Limestone continues to predominate in the area, although outcrops of Millstone Grit can be found (Dunham, 1948). The region as a whole is used largely for agricultural and forestry purposes. Four sampling sites in this region (Table 3.03) occur within the altitudes of 180 - 350 m.

Table 3.03. Sampling sites in East Allendale.

name	stream-reach	grid ref.	date sampled
River East Allen	0081 - 30	NY 847489	18/05/81
" " "	0081 - 50	NY 844522	18/05/81
" " "	0081 - 95	NY 803583	18/05/81
River Allen	0286 - 05	NY 800593	18/05/81

3.24 Region IV: Derwent Valley

The Derwent Valley, situated in the northeast part of the orefield, has been the subject of several studies regarding heavy metal pollution (Harding, 1978; Harding & Whitton, 1978, 1981; Burrows, 1981; Harding et al., 1981). The region is used extensively (agriculture, forestry, recreation) particularly since the construction of the Derwent reservoir between 1960 and 1966 (Harding et al., 1981). The principal village in the area is Blanchland, although the lower section (not studied) flows near Consett and Shotley Bridge. Sampling sites (Table 3.04) lie between 200 -300 m in altitude. Here the Upper Limestone series predominates, with Millstone Grit also present at the surface (Dunham, 1948). A fluorspar mine (previously worked for lead) at the head of Bolts Burn was the principal source of pollution, but activities have recently been suspended (Harding et al., 1981).

Table 3.04. Sampling sites in the Derwent Valley.

name	stream-reach	grid ref.	date sampled
River Derwent	0061 - 05	NY 956498	31/05/81
" "	0061 - 08	NY 959499	31/05/81
" "	0061 - 50	NZ 077503	31/05/81
Reeding Burn	0090 - 95	NY 958500	31/05/81

3.25 Region V: Weardale

Weardale here is defined as the upland valley of the River Wear from Wearhead to Wolsingham, including its tributaries and comprising altitudes from 200 to over 500 m. Stanhope and Wolsingham are the principal towns. Geological features in the area form an intrinsic part of the landscape and its industry, with an active fluospar mine and treatment works in operation near West Blackdene by British Steel, the Blue Circle Cement works in Eastgate and limestone quarries . Several smaller active fluorspar mines (previously lead mines) such as near Rookhope[1], Frosterley[1] and Cowshill, as well as many more disused mines are in evidence. Dunham (1948) records between 1666 and 1948 the greatest production of lead, iron and fluorspar of all the eight regions within the Northern Pennine Orefield. The region has an increasingly mixed geology on proceeding down the dale, from Great Limestone above, with Magnesian Limestone, Millstone Grit downstream and rocks of the Coal Measures outside of Wolsingham (Dunham, 1948). A total of 17 sites (Table 3.05) were sampled.

Table 3.05. Sampling sites in Weardale.

name	stream-reach	grid ref.	date sampled
River Wear	0008 - 07	NY 867392	10/05/81
" "	0008 - 09	NY 870387	10/05/81
" "	0008 - 17	NY 955385	13/05/81
" "	0008 - 40	NZ 075369	22/05/81
"Race Fell Burn"	0310 - 90	NY 918427	09/05/81
Daddryshield Burn	0309 - 95	NY 894379	10/05/81
Middlehope Burn	0307 - 98	NY 907381	10/05/81
Rookhope Burn	0012 - 45	NY 953386	13/05/81
Westernhope Burn	0306 - 95	NY 934378	13/05/81
Stanhope Burn	0320 - 95	NY 990394	13/05/81
Waskerley Beck	0123 - 50	NZ 067393	22/05/81
Thornhope Beck	0284 - 85	NZ 068378	22/05/81
Bollihope Burn	0015 - 15	NY 989352	22/05/81
" "	0015 - 18	NY 990352	22/05/81
" "	0015 - 98	NZ 039367	01/06/81
"Bollihope Cave Sike"	0305 - 01	NY 994352	22/05/81
Shittlehope Burn	0321 - 95	NZ 003385	18/05/81

1. operations ceased after completion of study.

3.26 Region VI: Durham Coalfield

This region of eastern county Durham includes the more populated regions of Birtley, Chester-le-Street and Durham city. Nine sites (Table 3.06) lie between 30 - 190 m in elevation. Industrial and sewage inputs are the principal sources of contamination in these rivers. However, Smith (1981) has recorded the presence of several mineral deposits (esp. Barium) within the region. The geology is predominated by the Coal Measures and Millstone Grit, with sandstones, shales and Magnesian Limestone (Dunham, 1948).

Table 3.06. Sampling sites in the Durham Coalfield.

name	stream-reach	grid ref.	date sampled
River Wear	0008 - 65	NZ 285409	17/05/81
River Deerness	0005 - 43	NZ 226422	17/05/81
River Browney	0014 - 14	NZ 165463	17/05/81
River Team	0024 - 03	NZ 168527	21/05/81
" "	0024 - 05	NZ 173529	21/05/81
" "	0024 - 20	NZ 202560	21/05/81
" "	0024 - 40	NZ 235543	21/05/81
" "	0024 - 56	NZ 258563	21/05/81
Tanfield Sewage Effluent	0360 - 01	NZ 197553	21/05/81

3.27 Region VII: Teesdale

Teesdale is a large upland region which includes the moorlands from Cow Green through Barnard Castle. It is less densely populated than adjacent Weardale and land is used mainly for grazing. Within the Upper, Middle and Lower Limestones, a great number of lead veins have been worked in the past (Dunham, 1948). However, elevated levels of heavy metals in the main river appear to be less than found in other mining regions of the Northern Pennines (Holmes & Whitton, 1981), such as the Wear. Millstone Grit is common in the vicinity of Barnard Castle (Ramsbottom et al., 1974). None of the mines are known to be active at present. Eight sites sampled (Table 3.07) range in altitude from 150 - 380 m.

Table 3.07. Sampling sites in Teesdale.

name	stream-reach	grid ref.	date sampled
Chester Sike	0355 - 95	NY 883302	01/06/81
Etters Gill	0354 - 98	NY 894283	01/06/81
Bow Lee Beck	0358 - 90	NY 907283	01/06/81
Stony Gill	0356 - 85	NY 931263	01/06/81
"Dry Level Sike"	0357 - 40	NY 939275	01/06/81
" " "	0357 - 45	NY 942273	01/06/81
Hudeshope Beck	0359 - 90	NY 947257	01/06/81
River Tees	0009 - 50	NZ 066149	01/06/81

3.28 Region VIII: Lower Tees

The lowland region of the River Tees is a more densely populated area, including the cities of Darlington and Stockton. Industrial and sewage inputs have an effect on the Tees, particularly via the calcareous River Skerne (Holmes & Whitton, 1981). The geology is predominantly Carboniferous Limestone with extensive marine deposits (Ramsbottom et al., 1974). Two sites (Table 3.08) were chosen to compare the river above and below the entry of the River Skerne.

Table 3.08. Sampling sites in the Lower Tees.

name	stream-reach	grid ref.	date sampled
River Tees	0009 - 65	NZ 270124	01/06/81
" "	0009 - 80	NZ 312101	01/06/81

3.29 Region IX: Arkengarthdale

The catchment of Arkle Beck is a rugged dale composed mainly of moorland and old lead mine remains. The small village of Langthwaite is the largest settlement in the dale itself, although at the stream's junction with the River Swale there is the larger village of Reeth. Much of the land is now used for grazing, even though parts of the land consist of old tailings or exposed limestone. The area is formed almost entirely of Carboniferous rocks, primarily from the Yoredale series of limestones and Millstone Grit (Ramsbottom et al., 1974). Mineralization is found throughout the area, particularly galena, zinc blende and fluorspar (Dunham, 1974). A long and colourful history of lead mining has been traced back to at least Roman times (Raistrick, 1975; Clough, 1980). An intensive period of mining continued for many centuries, particularly from the 17th through the 19th century, by the Charles Bathurst family, which lends its name (C.B.) to many mines, mills and landmarks. Several of the 12 sites (Table 3.09) are old hushes or adits associated with disused mines and smelting mills.

Table 3.09. Sampling sites in Arkengarthdale.

name	stream-reach	grid ref.	date sampled
Arkle Beck	0196 - 35	NY 983045	05/06/81
" "	0196 - 50	NZ 000035	05/06/81
" "	0196 - 60	NZ 005024	05/06/81
" "	0196 - 90	SE 041993	05/06/81
Great Punchard Gill	0276 - 90	NY 976050	05/06/81
Whaw Gill	0277 - 97	NY 982045	05/06/81
Fagger Gill	0303 - 90	NY 980050	05/06/81
"C.B. Gill"	0318 - 95	NZ 003027	05/06/81
Stang Burn	0315 - 98	NZ 000036	05/06/81
Hush Gutter	0278 - 75	NZ 003027	05/06/81
"Moor Hush Gill"	0279 - 98	NZ 004024	05/06/81
Booze Wood Level	0319 - 10	NZ 015020	05/06/81

3.210 Region X: Swaledale

This dale originates at the junction of Birkdale Beck and Great Sleddale Beck, about 3.5 km west of Keld. The uppermost of 13 sites (Table 3.10) is near Keld and the lowest is 100 m downstream of

Richmond Castle. The geology is based on the same Yoredale series as Arkengarthdale, but mineralization is more isolated in the Old Gang-Surrender and Gunnerside systems (Dunham, 1974). Hydrogeochemical studies in Arkengarthdale and Swaledale have shown aqueous metals are similarly localized (Ineson & Al-Badri, 1980). Farming and grazing are now the common landuses along the hillslopes and in the valley of the river itself.

Table 3.10. Sampling sites in Swaledale.

name	stream-reach	grid ref.	date sampled
Oxnop Gill	0314 - 98	SD 934976	03/06/81
Gunnerside Gill	0304 - 95	SD 951983	03/06/81
River Swale	0166 - 15	SD 950978	03/06/81
" "	0166 - 30	SE 046985	03/06/81
" "	0166 - 40	NZ 176009	03/06/81
Barney Beck	0198 - 95	SE 015987	03/06/81
Grinton Gill	0283 - 95	SE 047985	03/06/81
Cogden Gill	0197 - 50	SE 048969	03/06/81
" "	0197 - 90	SE 056982	03/06/81
Hags Gill	0316 - 90	SE 068973	03/06/81
Marske Beck	0282 - 75	SE 104004	03/06/81
Oxque Beck	0317 - 50	SE 079995	03/06/81
Badger Beck	0281 - 95	SE 167997	03/06/81

3.211 Region XI: Holme Valley

The River Holme originates in upland moorland and is regulated by several reservoirs. The river then flows through an industrialized region consisting primarily of textile mills. Principal towns along the segment of the river studied are Holmfirth, Brockholes and Honley. The three sampling sites (Table 3.11) occur at elevations between 150 - 200 m. The drainage from the Pennines is within the Millstone Grit series (known locally as Kinderscout Grit) and the river then continues into the Coal Measures (Bromehead _et al._, 1933). There few reports of mineralization, but heavy metal contamination is known to occur from industry and sewage (Brown, 1980).

Table 3.11. Sampling sites in the Holme valley.

name	stream-reach	grid ref.	date sampled
River Holme	0265 - 10	SE 127072	14/06/81
" "	0265 - 20	SE 145089	14/06/81
" "	0265 - 30	SE 150112	14/06/81

3.212 Region XII: Mersey Catchment

This sampling region west of the Pennines is a heterogeneous collection of rivers, all of which enter the River Mersey. Few generalizations can be made about the geology of the region as a whole except that the upper reaches of most streams drain moorland and are within the Millstone Grit series. Conditions change markedly downstream after passing through Coal Measures (Bromehead et al., 1933) and a variety of industrial developments. All eight sites studied (see below) are to some degree affected by industrial and/or sewage inputs. One river, the Etherow, has been shown to be contaminated by heavy metals and organic pollution (Say et al., 1981). Principal towns in the catchments include Glossop, Broadbottom and Chisworth (R. Etherow, Glossop Brook, Chisworth Brook), Delph, (R. Tame), and Littleborough (Hollingworth Lake Brook). Altitudes of the eight sites (Table 3.12) range between 90 - 150 m.

Table 3.12. Sampling sites in the Mersey catchment.

name	stream-reach	grid ref.	date sampled
"Hollingworth Lake Brook"	0274 - 80	SD 944142	13/06/81
River Tame	0261 - 20	SD 984079	13/06/81
River Etherow	0255 - 10	SK 016968	14/06/81
" "	0255 - 20	SK 013962	14/06/81
" "	0255 - 25	SK 004949	14/06/81
" "	0255 - 80	SJ 962908	14/06/81
Glossop Brook	0258 - 95	SK 011952	14/06/81
Chisworth Brook	0259 - 50	SJ 994923	14/06/81

3.213 Region XIII: Ribble Valley

This region, sometimes known as Ribblesdale, occurs within a broad valley whose river meanders through alluvial plains and whose solid geology consists of Carboniferous Limestone (Earp et al., 1961). In a few scattered localities mineralized fractures (lead and barytes) have been recognized. Principal towns are Clitheroe and Blackburn, whose industrial and sewage effluents have an effect on the water quality of rivers in the area (Harding, 1979). Three sampling sites (Table 3.13) lie between 30 - 50 m in elevation.

Table 3.13. Sampling sites in the Ribble valley.

name	stream-reach	grid ref.	date sampled
Mearley Brook	0275 - 95	SD 726405	13/06/81
River Ribble	0262 - 60	SD 716387	13/06/81
" "	0262 - 65	SD 687368	13/06/81

3.214 Region XIV: Lake District

This mountainous region encompasses the central and southern lakes from Keswick and Ullswater, south nearly to Kendal. The geology of the majority of the region is dominated by the Skiddaw Slate and Borrowdale Volcanic series, although Borrow Beck itself is within the Silurian complex of mudstones, shales and sandstones (Prosser, 1977). Streambeds typically consist of a mixture of resistant rocks; slates, granite and schists. There is a variety of mineral deposits and mines within the region, especially lead and copper (Eastwood, 1921). One mine is directly connected with one of the sampling sites (Table 3.14); the Greenside lead mine near Glenridding. This ceased operation in 1962, but has had a continued effect on Red Tarn Beck and Ullswater (Welsh & Denny, 1980).

Table 3.14. Sampling sites in the Lake District.

name	stream-reach	grid ref.	date sampled
Borrow Beck	0300 - 40	NY 549042	25/05/81
" "	0300 - 45	NY 553039	25/05/81
Pasture Beck	0290 - 95	NY 406132	25/05/81
Goldrill Beck	0291 - 50	NY 403134	25/05/81
Deepdale Beck	0296 - 90	NY 348143	25/05/81
Red Tarn Beck	0292 - 98	NY 389170	25/05/81
River Glendaramackin	0293 - 99	NY 315247	25/05/81

3.3 Details of seasonal sampling sites

Seven sites (Table 3.15) from the previous study were selected such that all could be sampled within one day. These exhibit a broad cross-section of physical and chemical features of rivers where aquatic bryophytes are found.

Table 3.15. Sampling sites for the seasonal survey.

site name	stream and reach no.	region
"High Crag Burn"	0101 - 05	I: Alston Moor
Lee Springs	0289 - 98	II: West Allendale
River West Allen	0085 - 50	II: West Allendale
"Race Fell Burn"	0310 - 90	V : Weardale
River Team, Kyo Heaugh	0024 - 05	VI: Durham Coalfield
River Team, Causey Arch	0024 - 20	VI: Durham Coalfield
River Wear, Shincliffe	0008 - 65	VI: Durham Coalfield

These include upland and lowland reaches, sites with and without metal contamination and a wide range of nutrient regimes. Detailed descriptions follow; grid references of each can be found in section 3.2.

3.31 "High Crag Burn"

This is a small groundwater-fed stream (Fig. 3.02) and drains old lead mines at High Hundybridge Level and the Cowper Dyke Heads Mine, both disused (Dunham, 1948). The exact location is 5 - 15 m downstream of the roadbridge. Tailings from past activities can still be seen about 250 m ENE of the site (Fig. 3.03), which reportedly contain quantities of sphalerite in addition to lead ores (Dunham, 1948). The streambed is a cascading series of limestone bedrock blocks which are almost entirely covered by Rhynchostegium riparioides. The average width is 1.0 m and the water carries no obvious peat colouration. The stream is shaded during the summer, but the general area is open moorland which is used for sheep grazing.

3.32 Lee Springs

The entire length of Lee Springs is about 30 m and the stream itself averages 1.5 m in width (Fig. 3.04). The stream apparently originates underground from several active springs, forming a pool (Fig. 3.05). This is then channelled under the roadbridge and contributes an obviously greater volume of water to Mohope Burn than the burn itself (Fig. 3.06). The reach is 0 - 10 m downstream of the roadbridge. There are old shafts and mines in the vicinity, but none are known to affect this site. The stream has a rich flora of aquatic bryophytes, angiosperms and macrophytic algae. Located in open grassy moorland, the stream is unshaded. Immediate land use in the area is primarily cattle and sheep grazing.

3.33 River West Allen, Blackpool Bridge

The West Allen is a medium-sized upland stream (Fig. 3.07) which is

Figure 3.02. "High Crag Burn," reach 0101-05, showing bedrock blocks covered with Rhynchostegium.

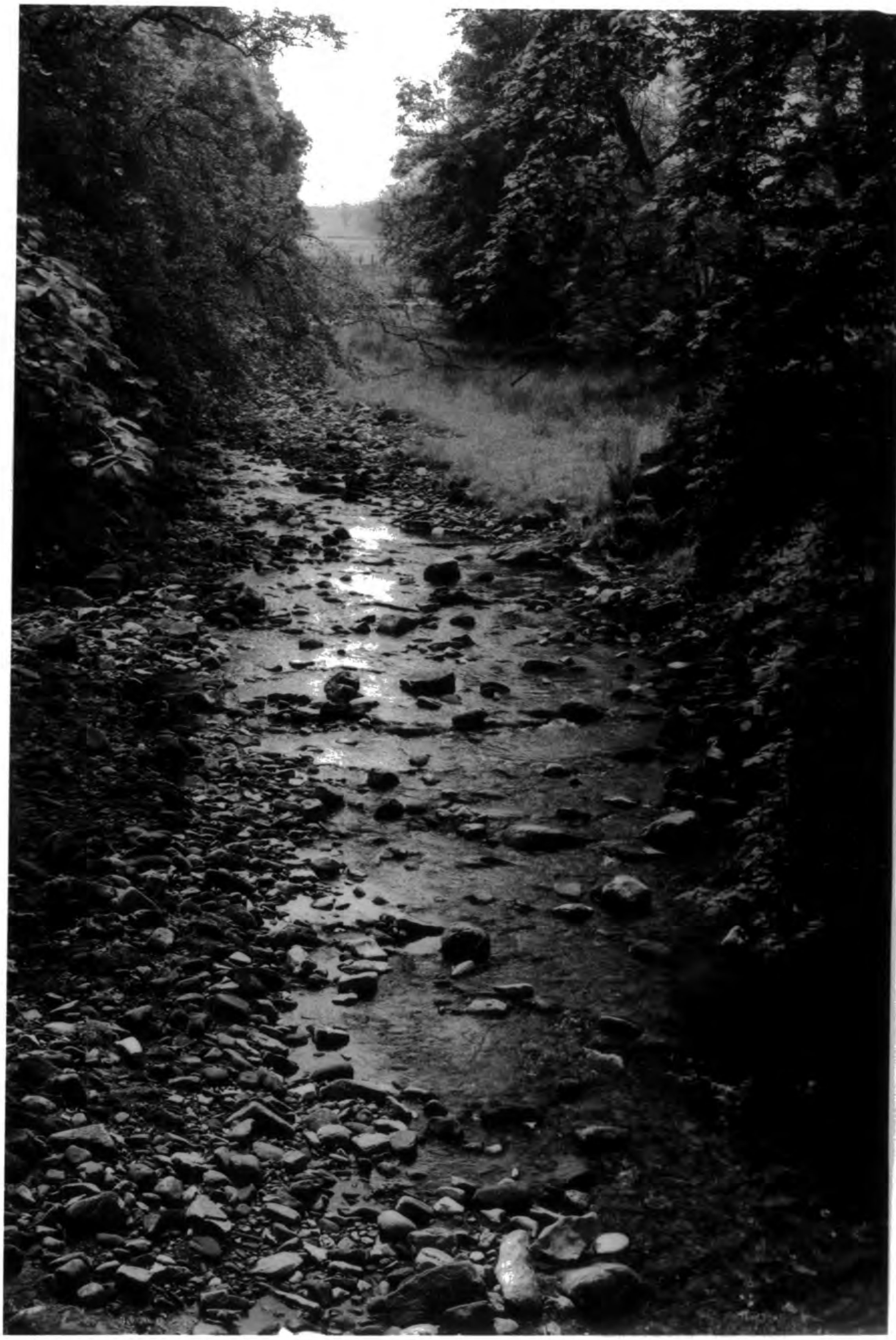
Figure 3.03. Waste lead tailings, Cowper Dyke Heads Mine, from which "High Crag Burn" drains.

Figure 3.04. Lee Springs, reach 0289-98.

Figure 3.05. Lee Springs, at spring source.

Figure 3.06. Entry of Lee Springs into Mohope Burn.

Figure 3.07. River West Allen, reach 0085-50.



influenced by a number of old lead mines along its length. The exact location this reach is 50 - 60 m upstream of the roadbridge. Principal mines (disused) are located at Coalcleugh and Carrshield (Dunham, 1948). Near the latter, tailings and waste can be seen along the banks of the river. The stream is approximately 10 m wide at this reach and the water is frequently coloured brown with humic acids. Much of the river is shaded during summer by several large trees. Bryophyte cover is somewhat sparse at this reach, as apparently many of the boulders are moved during high flows.

3.34 "Race Fell Burn"

This small upland stream (Fig. 3.08) is a tributary of Rookhope Burn, about 2 km west of Rookhope village. The area is heavily mineralized, with mines at West Groverake[1] and Redburn. The stream itself does not pass through any veins or old mines, however, and drains from a small plantation. The reach is largely unshaded, except for herbaceous vegetation. The reach is a small waterfall over limestone and grit boulders which are covered with the <u>Rhynchostegium</u> (Fig. 3.09). The reach begins 2 m above the waterfall and ends 8 m below. The stream averages only about 0.5 m in width and the water is uncoloured.

3.35 River Team, Kyo Heaugh

Much of the historical and geological background of the River Team has been described previously (Wehr <u>et al</u>., 1981) so only a brief summary is given here. This section of the river averages about 1.5 m in width (Fig. 3.10) and originates from an iron-rich spring associated

1. ceased operation after completion of study.

Figure 3.08. "Race Fell Burn," showing entry into Rookhope Burn.

Figure 3.09. "Race Fell Burn," reach 0310-90, showing waterfall covered with Rhynchostegium.

Figure 3.10. River Team, Kyo Heaugh, reach 0024-05.

Figure 3.11. River Team, Causey Arch, reach 0024-20.

with the Coal Measures. The reach is the last 10 m above the bridge of the minor road between West Kyo and Tantobie. The site is affected by an old domestic sewage system, but is upstream of industrial inputs of heavy metals. The aquatic vegetation is usually sparse.

3.36 River Team, Causey Arch

The reach at Causey Arch is located within a deep gorge formed by the diversion of the river around steep limestone cliffs known as Causey Ridge, which causes at least moderate shading year round. The location is 100 m downstream of the arch. The stream here is approximately 3 m wide and water is typically very cloudy (Fig. 3.11). Three effluents enter the river approximately 2 km upstream; a heavy metals input from a batteries factory, pumped groundwater from a nearby colliery and sewage from the Tanfield treatment works.

3.37 River Wear, Shincliffe

This broad, lowland site averages about 25 m in width and includes one large riffle (Fig. 3.12). The location of this reach is 20 - 30 m upstream of the A177 roadbridge. The river receives sewage from several sewage works in the vicinity and indirectly from nutrient-rich tributaries (Rivers Deerness, Browney, Gaunless). It is shaded only near the bank. The land use in the immediate area is residential and agricultural. A particularly rich macrophyte flora, consisting of several algal, bryophyte and angiosperm species is found here.

Figure 3.12. River Wear, Shincliffe, reach 0008-65.

CHAPTER 4. INTENSIVE RIVER SURVEY OF FIELD POPULATIONS OF RHYNCHOSTEGIUM RIPARIOIDES

4.1 Introduction

This chapter presents the results from an intensive survey of streams and rivers where the moss Rhynchostegium riparioides was found. This species was chosen for intensive surveys, as preliminary surveys and previous work have shown this species to be one of the most ubiquitous. Results for other aquatic mosses are given for comparison on a seasonal basis in chapter 5. The present survey of 105 sites was completed in a period of approximately six weeks from 6 May to 14 June 1981.

In section 4.2 the results for physicochemical variables and aqueous metals of all sites are presented. The transformation of these variables (for normalization) follows, which was a prerequisite to all statistical analyses which assume a normal distribution (Elliot, 1977). Correlations between these environmental variables complete this section. In section 4.3 the species of aquatic bryophytes from this survey are listed, including information on the abundance of Rhynchostegium. Metal concentrations in apical tips and whole plants of Rhynchostegium are presented in section 4.4. These data also are used to describe "baseline" concentrations expected in mosses from unpolluted sites. Normalization of these data is also conducted for the statistical sections to follow (4.5, 4.6).

Sections 4.5 and 4.6 introduce multivariate approaches to the analysis of data. An ordination of Rhynchostegium sites using principal components analysis (PCA) is given in section 4.5. Individual regions and sites are also compared using this approach. In section 4.6, an analysis of factors affecting metal accumulation by in situ field populations is presented. Simple linear correlations are first considered, followed by a stepwise multiple regression to consider what factors may have affected the accumulation of selected metals.

4.2 Physicochemical Characteristics and Aqueous Metals.

4.21 Physicochemical characteristics

During the survey, sites were sampled in a six week period when no sites were experiencing extremes of flow. Water samples from the 105 stream and river sites were analysed for 17 physicochemical variables (Appendix 1). The summarized data (Table 4.01) show the wide range of chemistries (particularly ammonia, nitrate and filtrable reactive phosphate) measured. The waters themselves varied markedly in the amount of colouration they exhibited, as evidenced by measurements of optical density (O.D.). Highest O.D. values at U.V. wavelengths (240, 254 nm) were recorded predominantly in streams with humic acids with peat drainage, such as Arkle Beck (e.g. 0196-35). However, some cloudy waters with apparently no humic colouration, such as the River Team (e.g. 0024-20) had moderately high O.D., comparable to upland sites on the River Wear (e.g. 0008-07) or Bollihope Burn (0015-15), making interpretation of O.D. values difficult.

The pH spectrum for Rhynchostegium sites was fairly broad, although most sites were alkaline. Seven sites had pH values ≤ 7.0, while 30 sites had measurements > pH 8.0. Low levels of total alkalinity (< 0.5 meq l^{-1}) and pH (< 7.0) were measured in streams of the Lake District and upstream sites on the River Etherow, Greater Manchester. While

Table 4.01 Descriptive statistics for physicochemical variables in 105 Rhynchostegium sites from intensive survey (no. = number detectable).

variable	units	no.	minimum	maximum	mean	standard deviation
temperature	^{o}C	105	6.5	17.5	12.1	2.2
O.D. 240 nm		105	0.001	1.101	0.257	0.208
O.D. 254 nm		105	0.001	0.958	0.215	0.181
O.D. 420 nm		105	0.001	0.126	0.024	0.023
conductivity	$\mu S\ cm^{-1}$	105	44	784	258	159
pH		105	6.8	8.7		
total alk	$meq\ l^{-1}$	105	0.10	7.12	1.42	1.00
NH_4-N	$\mu g\ l^{-1}$	104	< 5.0	1990	96.4	303
NO_2-N	"	83	< 1.0	195	11.8	32.7
NO_3-N	"	105	7.5	31900	1360	3820
FR PO_4-P	"	94	< 1.5	3180	72.9	330
FO PO_4-P	"	100	< 3.0	700	28.7	82.2
F^-	$mg\ l^{-1}$	105	0.025	1.30	0.26	0.29
Si	"	105	0.64	9.9	2.42	1.54
SO_4-S	"	105	1.30	80.0	12.1	17.2
Cl	"	105	5.2	155	18.1	20.5
O_2	% saturation	105	61	119	102	7.0

most low pH sites also had low total alkalinity, sites with higher pH were not always strongly buffered. The maximum total alkalinity recorded in the survey (7.12 meq l^{-1}) was for the River Deerness (pH 8.6), but the majority of sites with a pH > 8.0 had total alkalinities less than 2.0 meq l^{-1}. Those sites with low nitrate and phosphate concentrations were widely distributed, but were primarily upland streams located in the Lake District, Weardale, Teesdale and Swaledale. Lowland sites were as a group, more rich in these nutrients, particularly the lower sites on the Wear and Team, and the Tanfield sewage effluent. A total of 22 sites had concentrations of nitrate-N > 1.0 mg l^{-1}, but a concentration > 1.0 mg l^{-1} filtrable reactive phosphate-P was found in only one site.

Oxygen saturation was comparatively high throughout most of the sites sampled. Nearly all were saturated or supersaturated. Two types of river were undersaturated. These were organically polluted rivers (e.g. Team, 0024-20) and spring-fed streams with large amounts of precipitated iron present on the stream bottom (Booze Wood Level, 0319-10).

4.22 Aqueous metals

Results of metal analyses (Appendices 2, 3) are summarized in Table 4.02. Concentrations of several "non-heavy" metals (i.e. class A: sensu Nieboer & Richardson, 1980) such as sodium and potassium spanned more than one order of magnitude amongst sites. Concentrations were highest in those sites which also had elevated concentrations of nitrate and phosphate (e.g. lower R. Wear, Rivers Deerness & Team). Calcium and magnesium concentrations spanned more than one order of magnitude, with extremes (e.g. Lake District v. R. Deerness) of the two coinciding. Unlike the anions, calcium and magnesium were also fairly

Table 4.02 Descriptive statistics for metals in streamwater in 105 Rhynchostegium sites during intensive survey. Concentrations in mg l^{-1} (* = increased detection limit from concentrated samples; ! = too few detectable samples for calculation of mean and standard deviation).

element		no. detectable	minimum	maximum	mean	standard deviation
Na	T	105	2.6	89.0	12.5	16.6
	F	105	2.6	87.8	12.4	16.4
Mg	T	105	0.72	61.6	6.40	8.75
	F	105	0.72	60.0	6.29	8.57
K	T	105	0.08	15.2	2.10	2.56
	F	105	0.08	14.8	2.04	2.49
Ca	T	105	3.72	90.4	32.8	20.5
	F	105	3.72	88.0	32.4	20.2
Cr	T *!	27	< 0.001	0.16		
	F !	1	< 0.01	0.12		
Mn	T	103	< 0.004	0.77	0.060	0.110
	F	98	< 0.004	0.75	0.049	0.103
Fe	T	103	< 0.02	1.15	0.29	0.23
	F	99	< 0.02	0.58	0.14	0.12
Co	T *	105	0.002	0.017	0.006	0.004
	F !	0		< 0.02		
Ni	T *	105	0.003	0.070	0.015	0.009
	F !	0		< 0.04		
Cu	T *	105	0.0009	0.027	0.0047	0.0036
	F !	5	< 0.006	0.015		
Zn	T	100	< 0.006	1.75	0.138	0.253
	F	100	< 0.006	1.62	0.122	0.242
Cd	T	105	0.00009	0.00333	0.00058	0.00059
	F	105	0.00006	0.00332	0.00047	0.00052
Ba	T	105	0.05	0.91	0.26	0.21
	F	104	< 0.02	0.74	0.17	0.12
Pb	T	105	0.0010	0.200	0.018	0.030
	F	105	0.0010	0.178	0.011	0.025

high (e.g. Ca 40 mg l^{-1}) in several upland streams in Alston Moor, Weardale, Teesdale and Swaledale, which are limestone disticts. Overall, there were fewer (10 out of 105) extremely softwater sites, where calcium was < 10 mg l^{-1}.

Those metals which were mostly indetectable in the "filtrable" fraction (Cr, Co, Ni, Cu) were concentrated from "bulk" samples ten- or twentyfold by rotary evaporation prior to analysis. A test of the effectiveness of this method for three metals, calcium, manganese and zinc (Table 4.03), shows that the recovery was highly effective and

Table 4.03. Comparison of concentrations of "total" calcium, manganese and zinc in streamwater (0008-17; Sept. 1981) measured in unconcentrated and concentrated (via rotary evaporation) samples.

	Ca		Mn		Zn	
treatment	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
unconcentrated	18.9	0.54	0.076	0.004	0.089	0.004
concentrated	18.4	0.64	0.082	0.002	0.090	0.002

with good reproducibility. As a result of this step, cobalt, nickel and copper were detectable at all of the sites. Measurement of chromium was improved, but only 27 sites were within detectable concentrations. Elevated concentrations of chromium were measured at several sites, the highest in Chisworth Brook (0259-50; T = 0.16, F = 0.12 mg l^{-1}), which receives effluents from a chrome pigments factory (Harding, Say & Whitton, 1981). The remainder (Rivers Holme, Tees, Wear) were largely affected by sewage, although the River Etherow receives water from Chisworth Brook itself.

A wide range of heavy metal (Zn, Cd, Ba, Pb) concentrations were measured, barium being the least varied between sites. A total of 31 sites had filtrable zinc concentrations > 0.10 mg l^{-1} . The maximum concentration measured was in the River Nent (0048-90; T = 1.75, F = 1.62 mg l^{-1}); several other sites in Alston Moor also had comparatively high aqueous zinc. The Nent had the second highest cadmium measured (T = 2.59, F = 2.30 μg l^{-1}). Elevated concentrations of this metal were encountered in several other regions, particularly West Allendale, Arkengarthdale and Swaledale. Not all sources of cadmium were geological, however, as in the Mersey catchment (e.g. 0255-20; T = 0.52, F = 0.47 μg l^{-1}), the source was industrial. Generally, Lake District streams and upland sites in Teesdale had the lowest concentrations of most heavy metals.

Elevated concentrations of aqueous cadmium, barium and lead were located primarily in the Askrigg Block of the Northern Pennine Orefield. Barney Beck (0198-95) in Swaledale had the highest aqueous lead concentration (T = 0.200, F = 0.178 mg l^{-1} and the second highest barium concentration (T = 0.79, F = 0.66 mg l^{-1}). As was typical of many of the streams from these regions (Swaledale, Arkengarthdale), zinc was not much higher (T = 0.194, F = 0.174 mg l^{-1}). Streams from Alston Moor and other regions in the northern half of the orefield generally had 10 or more times zinc than lead. The highest aqueous cadmium for a Rhynchostegium site was measured in Booze Wood Level (0319-10; T = 3.33, F = 3.32 μg l^{-1}), also in Arkengarthdale.

4.23. Transformation and normalization of data

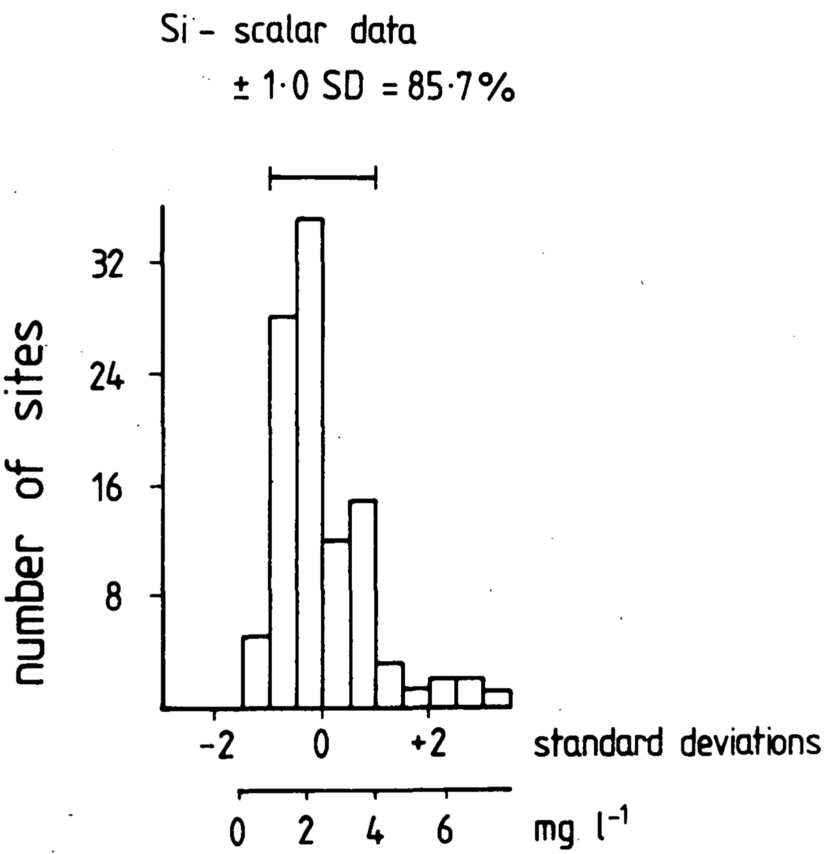
Prior to statistical analysis of water chemistry data, all variables were tested for normality. Two procedures were used to examine the distribution of each variable. The primary method was a distribution plot of the variable expressed as standard deviations from the mean. A normally distributed variable is distributed symmetrically about the mean and should tail off such that $\bar{x} \pm 1.0$ SD includes 68% of the observations and $\bar{x} \pm 2.0$ SD includes 95%. A sample distribution for silica (Fig. 4.01) shows this result. The untransformed (scalar) variable is negatively skewed (skewed to the left) and about 86% of all observations fall in the interval $\pm$ 1.0 SD.

The data were then tested after several common transformations: $\log_{10} x$, $\log_e x$, square root, $\log_{10}(x+1)$ and $\log_{10}(x/1-x)$; methods used by other workers with multivariate data (Statistical Research Laboratory, 1976; Green, 1979). These transformed variables were also plotted (e.g. Fig. 4.02). The resulting distribution after one of these transformations usually satisfied this first test of the normality assumption.

The second set of tests was the calculation of skewness (g1) and kurtosis (g2) coefficients (Snedecor & Cochran, 1967), which are numeric estimates of symmetry and peakedness, respectively, of a variable (Tables 4.04 & 4.05). When either are much greater than zero, normality is unlikely. Of the three most effective forms (scalar, square root and $\log_{10}$) for physicochemical variables (Table 4.04), only pH (already the $\log_{10}$ of $1/[H^+]$), temperature and percent O_2 saturation (a ratio) were most normally distributed in the scalar form. Distributions of optical density data were best improved using a square root transformation. All other physicochemical variables and aqueous metals (Table 4.05) were $\log_{10}$ transformed. For the majority of variables, skewness and kurtosis were reduced to < 1.0 by the appropriate transformation. The $\log_{10}$ transformation was most effective

Figure 4.01. Histogram of scalar distribution of sample variable (Si).

Figure 4.02. Histogram of $\log_{10}$ distribution of sample variable (Si).


Si - scalar data
± 1·0 SD = 85·7%
number of sites
32
24
16
8
-2
0
+2
standard deviations
0
2
4
6
mg l⁻¹

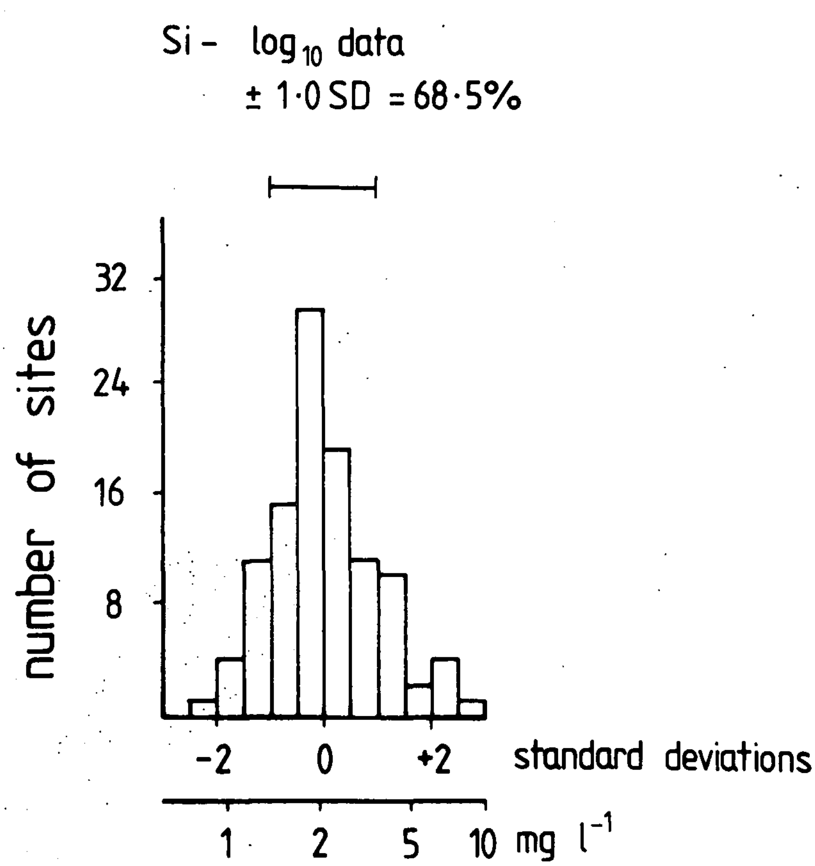

Si - log10 data
± 1·0 SD = 68·5%
number of sites
32
24
16
8
-2
0
+2
standard deviations
1
2
5
10
mg l⁻¹

Table 4.04 Comparison of skewness (g1) and kurtosis (g2) coefficients for measurements of physicochemical variables expressed as scalar (untransformed) values and both square root and $\log_{10}$ transformations (* = the form of the variable used for subsequent analyses; ! = too few cases for calculation).

variable	scalar g1	scalar g2	square root g1	square root g2	log 10 g1	log 10 g2
temperature	0.12	-0.17 *	-0.14	-0.07	-0.42	0.28
O.D. 240 nm	1.63	2.79	0.60	0.42 *	-2.26	9.62
O.D. 254 nm	1.69	2.96	0.67	0.43 *	-1.97	7.84
O.D. 420 nm	2.07	4.84	0.85	0.85 *	-0.75	0.76
conductivity	1.55	2.34	0.74	0.67	-0.26	0.48 *
pH	-0.25	-0.63 *	-0.31	-0.61	-0.36	-0.59
total alk	2.14	8.76	0.51	1.25	-0.82	-0.90 *
NH_4-N	5.07	25.8	3.54	13.5	1.66	2.32 *
NO_2-N	4.26	18.3	2.95	8.90	1.05	0.76 *
NO_3-N	5.71	38.8	3.05	11.1	0.64	0.74 *
reac PO_4-P	8.23	73.3	4.57	25.9	1.02	1.31 *
org PO_4-P	6.07	42.6	3.66	15.6	0.49	3.26 *
F^-	1.92	3.04	1.18	0.62	0.36	-0.74 *
Si	2.12	5.82	1.19	1.82	0.36	0.06 *
SO_4-S	2.91	7.34	2.23	4.50	1.03	1.22 *
Cl^-	3.78	18.7	2.27	6.06	1.33	1.24 *
% sat. O_2	1.78	10.7 *	-2.38	14.7	-3.04	19.7

Table 4.05 Comparison of skewness (g1) and kurtosis (g2) coefficients for measurements of aqueous metals expressed as scalar (untransformed) values and both square root and $\log_{10}$ transformations (* = the form of the variable used for subsequent analyses; ! = too few cases for calculation).

variable		scalar g1	scalar g2	square root g1	square root g2	log 10 g1	log 10 g2
Na	T	2.93	8.38	2.14	4.15	1.30	1.05 *
	F	2.92	8.31	2.14	4.15	1.32	1.08 *
Mg	T	4.16	19.4	2.58	8.21	0.70	1.64 *
	F	4.16	19.4	2.58	8.25	0.71	1.61 *
K	T	2.93	9.17	1.62	3.13	-0.12	0.59 *
	F	2.96	9.36	1.60	3.15	-0.23	0.74 *
Ca	T	1.09	0.82	-0.31	-0.11	-0.68	0.51 *
	F	1.07	0.74	-0.31	-0.14	-0.67	0.48 *
Cr	T !						
	F !						
Mn	T	3.96	18.5	2.37	6.12	0.75	0.78 *
	F	4.22	21.2	2.52	6.85	0.73	0.71 *
Fe	T	1.38	1.66	0.52	-0.09	-0.83	1.26 *
	F	1.49	2.14	0.55	-0.09	-0.54	0.19 *
Co	T	1.33	1.40	0.77	0.11	0.21	-0.54 *
	F !						
Ni	T	2.71	12.8	1.14	3.18	0.01	0.58 *
	F !						
Cu	T	2.67	12.2	1.11	2.17	0.18	-0.38 *
	F !						
Zn	T	4.30	21.8	1.97	5.14	0.13	-0.38 *
	F	4.39	22.2	2.16	5.86	0.33	-0.36 *
Cd	T	2.25	5.63	1.25	1.33	0.35	-0.55 *
	F	2.90	10.2	1.61	2.88	0.56	-0.21 *
Ba	T	1.36	1.04	0.81	-0.22	0.19	-0.78 *
	F	2.47	8.58	1.01	2.28	-0.42	1.37 *
Pb	T	3.99	18.9	2.02	5.24	0.38	-0.26 *
	F	5.59	33.4	3.19	13.2	0.81	0.78 *

in normalizing a majority of variables in this dataset. It must be emphasized that these tests indicate the distribution is a function of the sites sampled, not of the variables themselves. Another dataset may not have the same characteristics.

In statistical analyses of these data (the following sections) no mention will be made of the transformations used for each variable (e.g. $\log_{10}$ Si or square root O.D. 240 nm) as it is their normality which is of importance. Analyses which required normally distributed variables (e.g. correlation, principal components analysis, analysis of variance) are carried out using the most normal form achieved and will subsequently be referred to simply as "normalized," throughout this thesis.

4.24 Correlations between water chemistry variables.

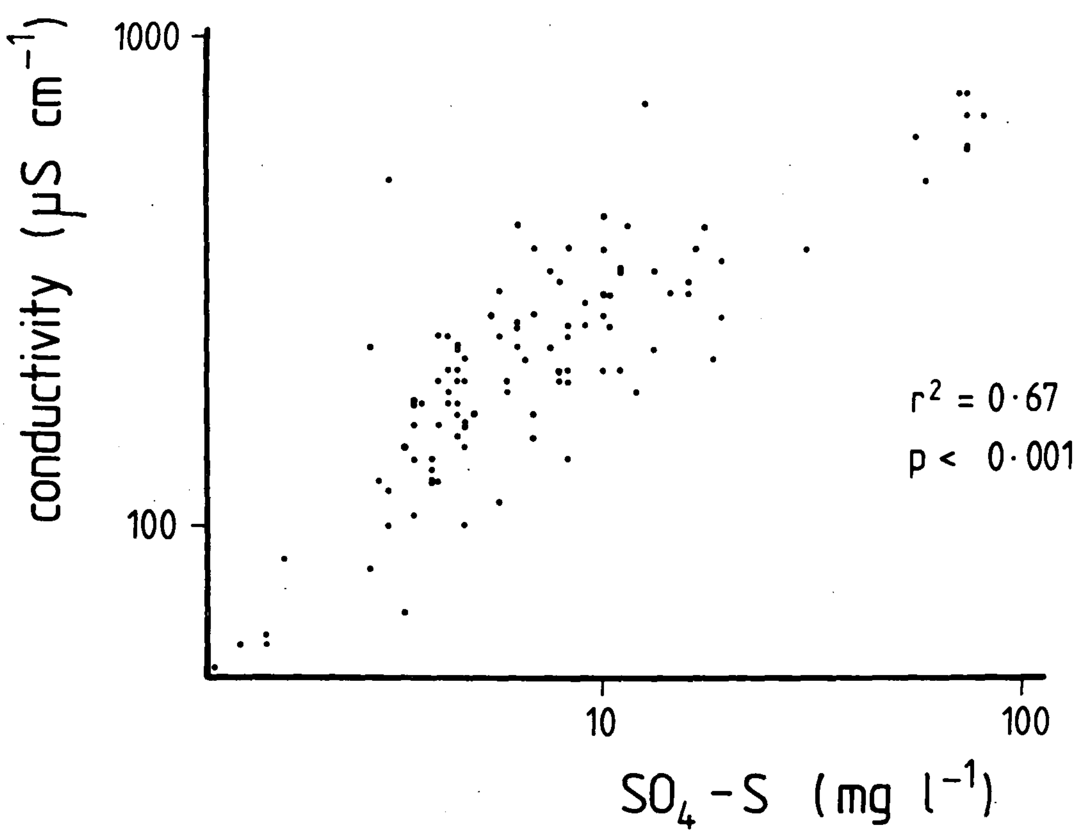
Several physical and chemical variables of the streams were apparently related in their minimum and maximum values and in the locations of these extremes (section 4.21). A further consideration of these data by correlation (Table 4.06) shows that many variables were intercorrelated. Conductivity, a rough measure of total ionic substances (Mackereth et al., 1978), was most strongly correlated with sulphate ($r = 0.82$, $p << 0.001$), but positive correlations also exist with total alkalinity, chloride, and filtrable reactive silica. A scattergram between sulphate concentrations and conductivity (Fig. 4.03) shows the relationship had few exceptions. Conductivity in these streams was negatively correlated (significantly) with optical density (O.D. 420 nm: $r = -0.36$, $p < 0.001$). Although previous observations (section 4.21) indicated that not all high alkalinity sites had higher pH values, there was still a significant correlation between these two

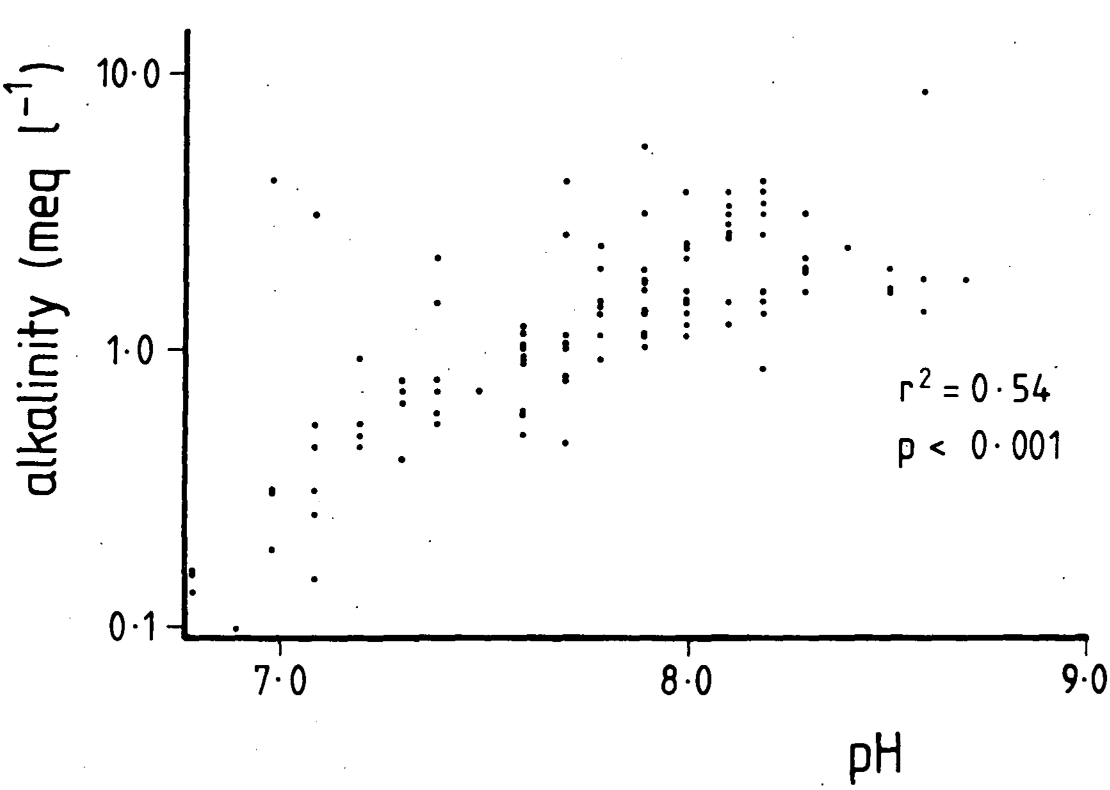
Table 4.06 Correlations between physicochemical variables of 105 Rhynchostegium sites from intensive survey (variables are transformed to normal distribution, see section 4.23; * = p < 0.05, ** = p < 0.01, *** = p < 0.001).

	temp	O.D. 240	O.D. 254	O.D. 420	cond	pH	tot alk	NH_4	NO_2	NO_3	FRP	FOP	F	Si	SO_4	Cl
temp																
O.D. 240	0.13															
O.D. 254	0.10	0.99***														
O.D. 420	0.02	0.94***	0.95***													
cond	0.22*	−0.24*	−0.28**	−0.36***												
pH	0.24*	−0.03	−0.03	−0.17	0.51**											
tot alk	0.01	−0.08	−0.10	−0.20*	0.80***	0.73***										
NH4	0.35***	−0.06	−0.14	−0.14	0.35***	−0.14	−0.05									
NO2	0.48***	0.19*	0.14	0.10	0.46***	0.04	0.13	0.67***								
NO3	0.15	−0.27**	−0.34***	−0.29**	0.56***	0.01	0.18	0.70***	0.62***							
F.R.PO4	0.46***	0.23*	0.17	0.12	0.37***	0.02	0.07	0.71***	0.84***	0.63***						
F.O.PO4	0.30**	−0.01	−0.01	−0.08	0.40***	0.02	0.14	0.64***	0.60***	0.58***	0.69***					
F	0.01	−0.22*	−0.21*	−0.29**	0.42***	0.47***	0.50***	−0.10	−0.05	−0.02	−0.09	0.01				
Si	0.17	−0.29**	−0.32***	−0.33***	0.59***	−0.01	0.29**	0.45***	0.39***	0.56***	0.36***	0.40***	0.15			
SO4	0.20*	−0.29**	−0.35***	−0.40***	0.82***	0.30**	0.56***	0.42***	0.51***	0.62***	0.44***	0.46***	0.38***	0.61***		
Cl	0.34***	−0.32***	−0.36***	−0.40***	0.66***	0.13	0.24*	0.52***	0.67***	0.70***	0.52***	0.49***	0.16	0.54***	0.71***	
% O2	0.14	0.15	0.17	0.11	−0.15	0.38***	0.01	−0.27**	−0.10	−0.27*	−0.12	−0.23*	−0.08	−0.44***	−0.15	−0.16
	temp	O.D. 240	O.D. 254	O.D. 420	cond	pH	tot alk	NH_4	NO_2	NO_3	FRP	FOP	F	Si	SO_4	Cl

Figure 4.03. Scattergram showing relationship between aqueous sulphate and conductivity in sites from *Rhynchostegium* surveys.

Figure 4.04. Scattergram showing relationship between total alkalinity and pH in sites from *Rhynchostegium* surveys.


1000
100
conductivity (µS cm⁻¹)
10
100
SO₄-S (mg l⁻¹)
r² = 0·67
p < 0·001


10·0
1·0
0·1
alkalinity (meq l⁻¹)
7·0
8·0
9·0
pH
r² = 0·54
p < 0·001

variables. An examination of a scatter between pH and alkalinity (Fig. 4.04) reveals that most, but not all sites fell along this line.

There were several significant correlations within another group of variables, including ammonia, nitrite, nitrate and both forms of phosphate. All of these "nutrients" were positively correlated to one another at a significance level of $p < 0.001$. Oxygen saturation was highest in streams with decreased concentrations of these variables; there were significant negative correlations with three out of five of these. Relationships pointed out by many of these correlations are considered tentative. For example, water temperature (in part a function of sampling time and date) correlated significantly with as many as eight physicochemical factors.

Concentrations of aqueous metals in both the total (T) and filtrable (F) fractions were measured. With the exception of a few elements (Cr, Co, Ni, Cu), the majority of sites had detectable concentrations of metals so that concentrated samples were unnecessary. For these elements, correlations between the two fractions (Table 4.07)

Table 4.07 Correlations between total and filtrable concentrations of metals from 105 <u>Rhynchostegium</u> sites from intensive survey (* = $p < 0.001$; insufficient data for Cr, Co, Ni, Cu for calculation).

element	r
Na	0.99 *
Mg	0.99 *
K	0.99 *
Ca	0.99 *
Mn	0.90 *
Fe	0.78 *
Zn	0.98 *
Cd	0.89 *
Ba	0.78 *
Pb	0.85 *

indicate a strong relation for each element. The weakest two T:F correlations were for iron and barium, although the correlations were still highly significant ($p < 0.001$). This difference is considered further for metal accumulation (section 4.6). In light of these strong correlations, the correlations between different elements include only filtrable concentrations (as an estimate of "dissolved" metals), with the exception of the four elements which required concentration prior to analysis. Hence, whenever a symbol such as "Pb.wat" appears this will refer to the filtrable fraction unless stated otherwise.

Correlations between concentrations of aqueous metals (Table 4.08) indicate several relationships. Positive correlations were found between sodium, magnesium, potassium, calcium and manganese. Scattergrams of sodium v. potassium (Fig. 4.05) and calcium v. magnesium (Fig. 4.06) illustrate this relationship. Cobalt and nickel were apparently allied to this group, although closer inspection of the entire matrix reveals that these two correlate fairly strongly with nearly every metal. The heavy metals (here Zn, Cd, Ba, Pb) were intercorrelated and the scattergram of zinc and cadmium (Fig. 4.07) is one example. Some heavy metals, notably barium and zinc, were also correlated positively with elements such as potassium and magnesium. In contrast, there was a significant negative correlation between lead and sodium. Too few chromium concentrations were measurable (27 out of 105) for this element to be included.

4.3 Species of aquatic bryophytes from intensive survey

4.31 Distribution and abundance of Rhynchostegium

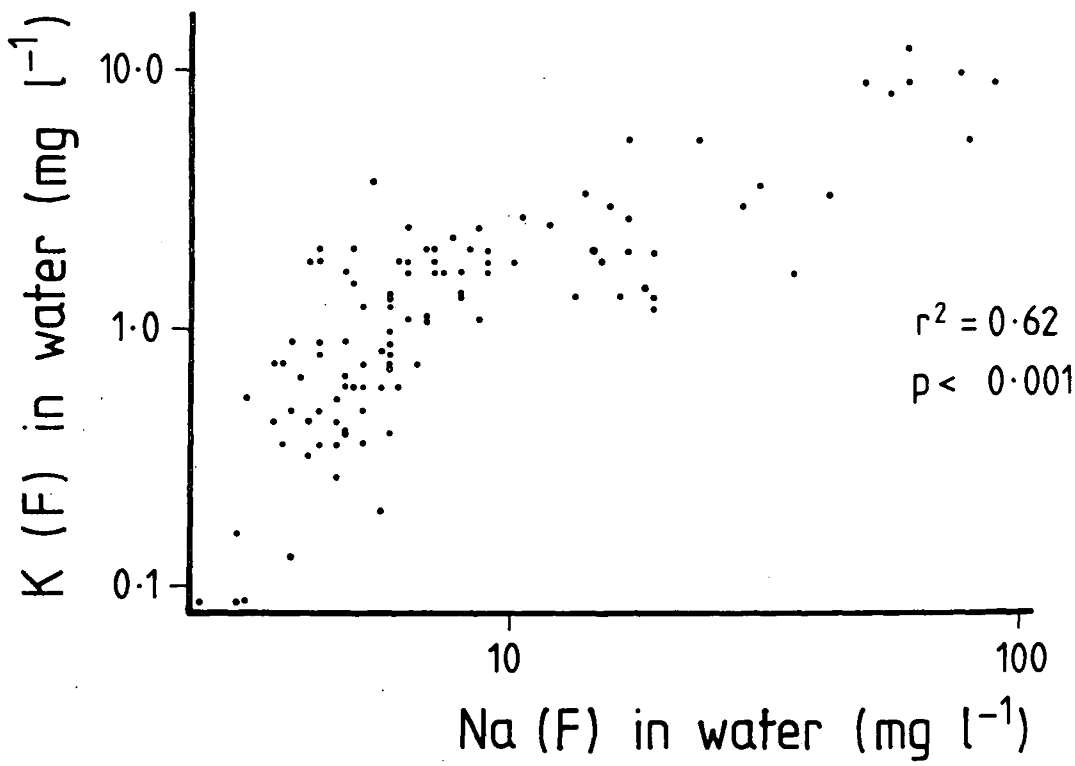
Rhynchostegium varied in abundance and cover among the sites

Table 4.08 Correlations between concentrations of aqueous metals from 105 *Rhynchostegium* sites from intensive survey (based on filtrable (F) metals except where insufficient cases prevent calculation (T), see Table 4.02; data are transformed to normal; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

	Na.F	Mg.F	K.F	Ca.F	Mn.F	Fe.F	Co.T	Ni.T	Cu.T	Zn.F	Cd.F	Ba.F
Na.F												
Mg.F	0.74***											
K .F	0.79***	0.83***										
Ca.F	0.48***	0.77***	0.67***									
Mn.F	0.69***	0.60***	0.62***	0.34***								
Fe.F	0.08	0.11	0.15	0.09	0.28**							
Co.T	0.52***	0.63***	0.52***	0.67***	0.43***	0.01						
Ni.T	0.58***	0.68***	0.63***	0.73***	0.50***	0.06	0.63***					
Cu.T	0.42***	0.35***	0.36***	0.41***	0.45***	0.33***	0.38***	0.61***				
Zn.F	0.09	0.29**	0.38***	0.43***	0.26**	0.02	0.19*	0.32***	0.22*			
Cd.F	-0.09	0.06	0.13	0.21*	0.10	-0.04	0.10	0.21*	0.17	0.74***		
Ba.F	0.07	0.33***	0.26**	0.65***	0.03	0.21*	0.38***	0.42***	0.40***	0.33***	0.30***	
Pb.F	-0.32***	-0.14	-0.12	0.08	-0.14	0.08	-0.08	-0.02	0.17	0.40***	0.45***	0.34***
	Na.F	Mg.F	K.F	Ca.F	Mn.F	Fe.F	Co.T	Ni.T	Cu.T	Zn.F	Cd.F	Ba.F

Figure 4.05. Scattergram showing relationship between aqueous K and Na in sites from Rhynchostegium surveys.

Figure 4.06. Scattergram showing relationship between aqueous Ca and Mg in sites from Rhynchostegium surveys.


10·0
1·0
0·1
10
100
K (F) in water (mg l^{-1})
Na (F) in water (mg l^{-1})
$r^2 = 0{\cdot}62$
$p < 0{\cdot}001$

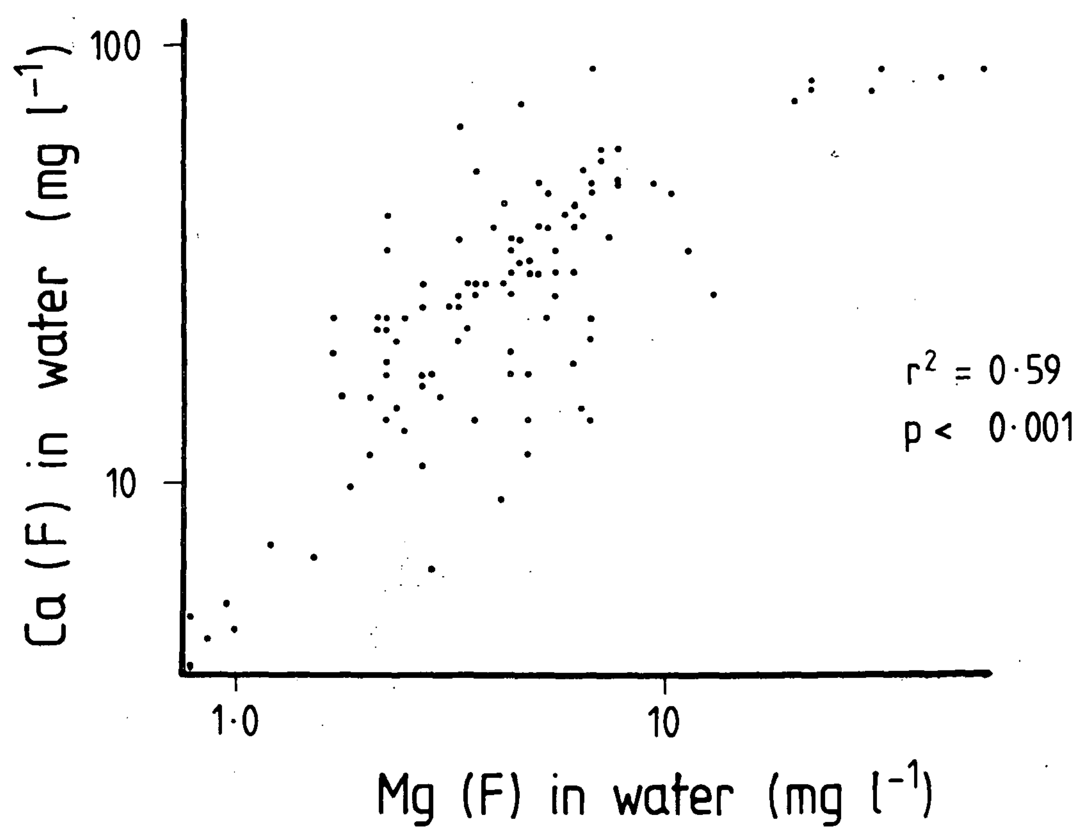
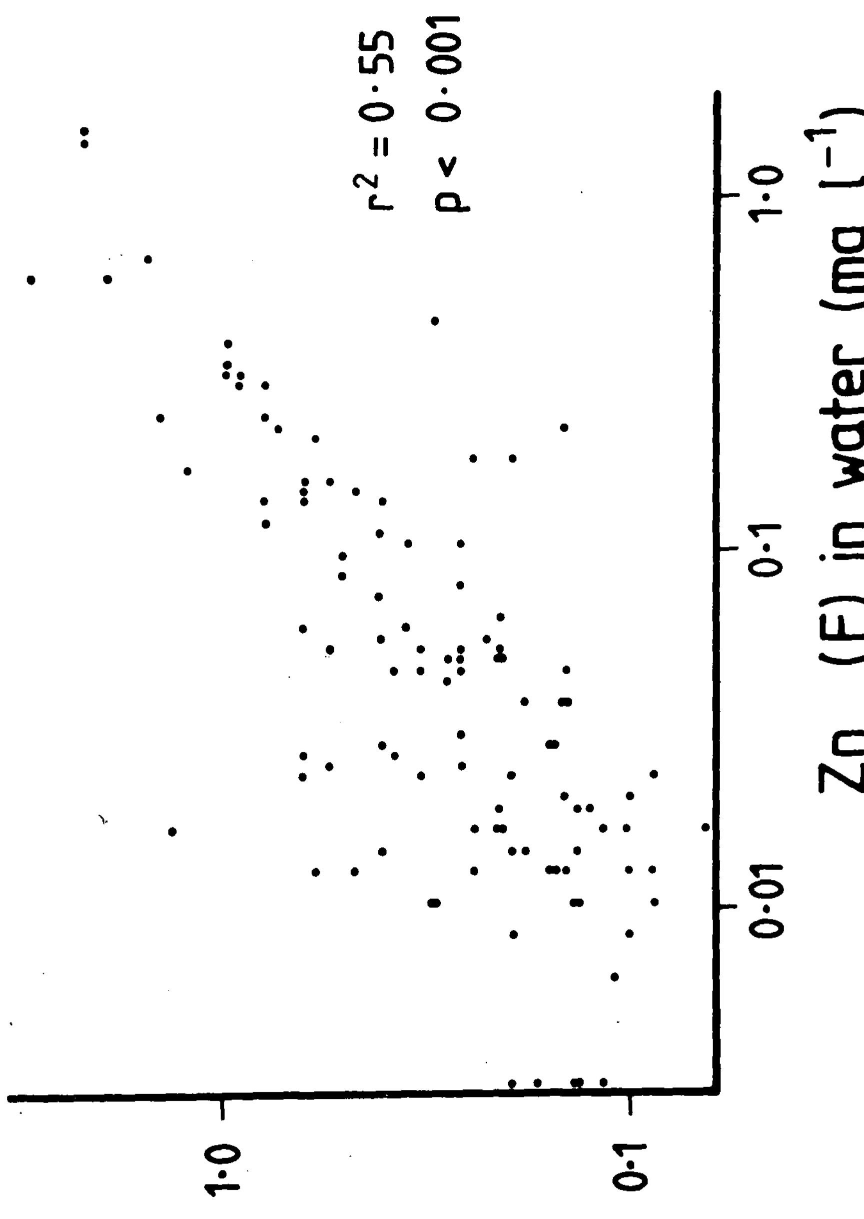

100
10
1·0
10
Ca (F) in water (mg l^{-1})
Mg (F) in water (mg l^{-1})
$r^2 = 0{\cdot}59$
$p < 0{\cdot}001$

Figure 4.07. Scattergram showing relationship between aqueous Cd and Zn in sites from Rhynchostegium surveys.


$r^2 = 0.55$
$p < 0.001$
Zn (F) in water (mg l^{-1})
Cd (F) in water (μg l^{-1})
0·01
0·1
1·0
0·1
1·0

sampled. A summary of these findings (Table 4.09) indicates that this

Table 4.09. Relative abundance and percent cover of Rhynchostegium at sites from the intensive survey, giving the percentage of sites with each amount (relative abundance after Holmes & Whitton, 1977a; see Methods).

relative abundance	sites n	sites %	percent cover	sites n	sites %
1 = rare	1	0.9	< 1.0 %	31	30
2 = occasional	7	6.7	1.0 - 5.0 %	33	31
3 = frequent	13	12	5.0 - 10 %	16	15
4 = abundant	17	16	10 - 30 %	18	17
5 = very abundant	67	64	30 - 50 %	2	1.9
			50 %	5	4.8

species was a dominant macrophyte at a majority of the streams sampled. There was bias, however, in that sampling was less likely to be carried out where the species was present but difficult to find. Nonetheless, it was not uncommon to observe streams where nearly the only aquatic moss present was Rhynchostegium, often covering a large proportion of the stream bed. A total of 41% of all sites sampled had more than 5% cover by this species. There was no obvious pattern in the abundance or cover of Rhynchostegium amongst various kinds of sites. Typically, large masses of this species were observed in springs, but frequently in larger streams as well. Nor was abundance related to water quality. The Tanfield Sewage effluent (0360-01) in county Durham and "Race Fell Burn" (0310-90) in upland Weardale each had 75% cover by Rhynchostegium. "High Crag Burn" (0101-05) with elevated zinc, cadmium and lead had 60% cover while in uncontaminated Chester Sike (0355-95), nearly 80% of the streambed was covered by this moss. Further, in streams where Rhynchostegium was the dominant macrophyte, percent cover was not always high.

Table 4.10. List of all aquatic bryophyte species (including three macroalgae) identified from the intensive survey of Rhynchostegium sites, with the number (n) and percentage (%) of sites from the total of 105 (* = a complex of two species: B. moniliforme & B. ectocarpum).

species	n	%
macrophytic algae		
Batrachospermum spp. *	15	14
Cladophora glomerata (L.) Kütz.	30	28
Lemanea fluviatilis (L.) Agardh.	54	51
bryopytes - liverworts		
Chiloscyphus polyanthos (L.) Corda.	3	2.8
Scapania undulata (L.) Dum.	14	13
Solenostoma triste (Nees) K. Mull.	1	0.9
bryophytes - mosses		
Amblystegium fluviatile (Hedw.) Br. Eur.	4	3.8
A. riparium (Hedw.) Br. Eur.	11	10
Brachythecium rivulare Br. Eur.	15	14
Bryum pallens Sw.	1	0.9
Cinclidotus fontinaloides (Hedw.) Beauv.	14	13
C. mucronatus (Brid.) Mach.	25	24
Dichodontium pellucidum (Hedw.) Schimp.	1	0.9
Fissidens crassipes Br. Eur.	3	2.8
Fontinalis antipyretica Hedw.	34	32
F. squamosa Hedw.	8	7.6
Hygrohypnum luridum (Hedw.) Jenn.	19	18
H. ochraceum (Wils.) Loeske	31	30
Rhizomnium punctatum (Hedw.) Kop.	2	1.9
Rhynchostegium riparioides (Hedw.) C. Jens.	105	100
unidentified pleurocarp	1	0.9

4.32 Other aquatic bryophytes

Other aquatic bryophytes were recorded during this survey (Table 4.10), including three liverworts and 14 mosses. Those most frequently encountered were Fontinalis antipyretica in lowland rivers and Hygrohypnum ochraceum and Cinclidotus mucronatus in upland streams. During preliminary surveys many upland streams lacking Rhynchostegium were found to have either H. ochraceum, H. luridum or Scapania undulata present. Few mid to lowland rivers were without Rhynchostegium. In some regions, this species was less common in small upland becks and gills (e.g. East Allendale, Alston Moor), than in others (esp. Swaledale, Arkengarthdale) where it was found in nearly every stream examined. Of all the "macrophytes" (sensu Holmes & Whitton, 1977a) which occurred with R. riparioides, the red alga Lemanea was by far the most common. Some bryophytes were patchily distributed. Fontinalis squamosa was observed in sites (from intensive survey) only west of the Pennines. A few streams lacking Rhynchostegium in the moors between upper Teesdale and upper Swaledale did have F. squamosa, however. Cinclidotus fontinaloides was most common in larger rivers of limestone districts (e.g. Rivers Wear, Swale, Ribble).

4.4 Concentrations of metals in populations of Rhynchostegium

4.41 Descriptive statistics

Concentrations of 14 metals were measured in both tips and whole plants of Rhynchostegium (Appendices 4, 5: pp. 427-432). Through the use of higher dry weights ($\bar{x}$ tips = 222 mg; $\bar{x}$ whole = 280 mg), all metals analysed were detectable in the digests; data are summarized in Table 4.11. On average, potatassium, calcium and iron concentrations in the tips were higher than other metals, while manganese and

Table 4.11. Descriptive statistics for metal concentrations ($\mu g\ g^{-1}$) in 2 cm tips and whole plants of Rhynchostegium from intensive survey of 105 sites.

element fraction	minimum	maximum	mean	standard deviation	$\bar{x}$ [tip] / $\bar{x}$ [whole]
Na tips	183	861	477	154	
whole	225	789	436	128	1.09
Mg tips	853	3440	1870	509	
whole	756	3730	2020	579	0.92
K tips	2780	12300	6280	1990	
whole	2340	10300	5900	1670	1.06
Ca tips	3010	30400	12100	4070	
whole	3800	57100	20600	7760	0.59
Cr tips	1.61	647	16.4	68.8	
whole	2.34	1330	28.3	136	0.58
Mn tips	108	18600	2890	3010	
whole	280	143000	15200	19400	0.19
Fe tips	1100	62600	8190	9190	
whole	1810	143000	14600	18700	0.56
Co tips	1.83	102	18.6	16.8	
whole	4.58	542	91.3	112	0.20
Ni tips	9.47	98.4	27.5	15.6	
whole	13.8	694	76.6	94.3	0.36
Cu tips	3.86	116	18.3	17.3	
whole	5.38	157	28.1	26.0	0.65
Zn tips	67.6	8840	1470	1640	
whole	128	22300	3290	3590	0.45
Cd tips	0.264	64.7	8.70	9.20	
whole	0.498	89.5	19.9	16.5	0.44
Ba tips	62.1	3550	527	675	
whole	126	3230	946	738	0.56
Pb tips	14.6	8690	677	1160	
whole	30.9	17800	1630	2560	0.42

iron were the most abundant metals in whole plants. Ranges of some metals, particularly manganese, zinc, cadmium, and lead spanned more than two orders of magnitude. Other metals, such as sodium and potassium varied little between sites.

A population from the River Nent (0048-90) had the highest zinc concentration in tips, which was also the site with the highest aqueous zinc. A total of 60 populations had zinc concentrations < 1000 $\mu g\ g^{-1}$ in tips, but only three were < 100 $\mu g\ g^{-1}$. The maximum cadmium measured in tips of Rhynchostegium was 64.7 $\mu g\ g^{-1}$, from Chisworth Brook, a site with elevated aqueous cadmium (T = 1.49, F = 0.62 $\mu g\ l^{-1}$), but less than several other sites. The second highest concentration in moss, 38.8 $\mu g\ g^{-1}$, was in the River Nent population, where the second highest aqueous cadmium was measured (T = 2.59, F = 2.30 $\mu g\ l^{-1}$). The maximum lead in moss tips (8690 $\mu g\ g^{-1}$) was measured in a population from Red Tarn Beck (0292-98), the Lake District, which drains from the Greenside lead mine, but was not the site of maximum lead in streamwater (T = 0.0795, F = 0.0590 $mg\ l^{-1}$). Sites with the three next highest lead concentrations in moss were located in Swaledale and Arkengarthdale (0278-75, 0319-10, 0198-95).

Concentrations of most elements were higher in whole plants. A pair-wise t-test between concentrations in these two plant fractions (Table 4.12) indicates differences were significant for 13 out of 14 elements. Only sodium and potassium were significantly higher in apical tips. The ratio of metal concentrations in whole v. tips ranged from 2 - 20; the average ratio was most extreme in manganese, whose concentrations were as high as 14% of the dry weight in whole plants.

Table 4.12. Comparison of mean concentrations of metals in tips and whole plants of Rhynchostegium via pairwise t-test (n = 105; mean difference in $\mu g\ g^{-1}$; p = significance; NS = non-significant).

element	mean difference	t-value	result	p
Na	41.0	2.68	tips > whole	< 0.01
Mg	155	- 6.04	whole > tips	< 0.001
K	385	3.57	tips > whole	< 0.001
Ca	8470	-18.3	whole > tips	< 0.001
Cr	11.9	- 1.78	tips = whole	NS
Mn	12300	- 4.01	whole > tips	< 0.001
Fe	6470	- 5.80	whole > tips	< 0.001
Co	72.7	- 7.42	whole > tips	< 0.001
Ni	49.1	- 5.79	whole > tips	< 0.001
Cu	9.8	- 7.66	whole > tips	< 0.001
Zn	1820	- 7.41	whole > tips	< 0.001
Cd	11.2	-10.4	whole > tips	< 0.001
Ba	419	- 7.84	whole > tips	< 0.001
Pb	950	- 5.53	whole > tips	< 0.001

4.42 Baseline concentrations

These data were also used to estimate what concentrations in plants may be considered the "baseline" (Hargreaves, 1981) for metals in uncontaminated rivers (particularly where aqueous concentrations were indetectable). The most obvious element for this approach was chromium. This metal was detectable in water from about a quarter of the sites (after concentration), but the plant data (Appendix 5: p. 430) indicate that several sites with unmeasured chromium in water had some contamination (e.g. the lower Tees: 0009-80). In 92 of the 105 sites, moss tips had < 10 $\mu g\ g^{-1}$ chromium. None of these sites were known to have any apparent sources for the metal and concentrations varied between about 2 and 10 $\mu g\ g^{-1}$ in various types of site. For example, plants from the River East Allen (0081-95), an upland site, had 8.75 $\mu g\ g^{-1}$ in tips, while plants from the organically polluted River Deerness (0005-43) contained only 2.93 $\mu g\ g^{-1}$.

Estimates of baseline concentrations of zinc, cadmium, barium and lead were made by examining the complete dataset (Appendix 5) and through ranking the data from all 105 sites (Table 4.13). Baseline estimates were also given as ranges, because of variabilty and the effects of other factors affecting accumulation (section 4.6). Zinc concentrations in <u>Rhynchostegium</u> tips were commonly between 100 - 1000 $\mu g\ g^{-1}$, but many of these were < 500 $\mu g\ g^{-1}$. This element did occur below that value in several types of site. Hence, the maximum value in uncontaminated waters ranged between 100 - 250 $\mu g\ g^{-1}$. The concentration range of cadmium was fairly well spread and plants from the majority of sites were below 10 $\mu g\ g^{-1}$. This resulted in a minimum range of 1 - 5 $\mu g\ g^{-1}$ cadmium in tips from sites which were uncontami-

Table 4.13. Ranges of zinc, cadmium, barium and lead concentrations ($\mu g\ g^{-1}$) in 2 cm apical tips of *Rhynchostegium*, indicating the number (n) and percentage (%) of sites for each and "baseline" estimates.

	$\leq$ 1.0		> 1.0 $\leq$ 10		> 10 $\leq$ 100		> 100 $\leq$ 1000		> 1000		estimated "baseline"
	n	%	n	%	n	%	n	%	n	%	
Zn	0	0	0	0	3	2.8	57	54	45	43	100 - 250
Cd	2	1.9	72	69	30	29	0	0	0	0	1 - 5
Ba	0	0	0	0	4	3.8	88	84	13	12	100 - 200
Pb	0	0	0	0	30	29	59	56	16	15	25 - 100

nated. Barium concentrations were less varied and exhibited less obvious groupings. An upper limit of 100 - 200 $\mu g\ g^{-1}$ in <u>Rhynchostegium</u> includes most of the sites. Lead concentrations in moss were commonly less than 100 $\mu g\ g^{-1}$ and few plants from uncontaminted sites had concentrations > 25 - 100 $\mu g\ g^{-1}$.

4.43 Transformation and normalization of data

Prior to statistical treatment of these data, tests were made for normality and appropriately transformed, as explained in section 4.23. Metals in plants, were as a group lognormally distributed (Table 4.14). A $\log_{10}$ transformation produced a more symmetric distributión, compared with scalar values. However, the distribution of some transformed variables, although improved, displayed a Cauchy distribution (i.e. more observations beyond the intervals $\pm$ 1.0 SD, so that the curve did not "tail off"). Results of $\log_{10}$ and square root transformations when tested for skewness and kurtosis show that $\log_{10}$ reduced these attributes in all variables, usually to < 1.0. The distribution of two variables (Ca in tips & whole plants) were only slightly improved through transformation (see also Appendix 4), but this is largely a problem of peakedness, not of being skewed. As with water chemistry data, these data will be referred to as "normalized" variables.

4.5 Comparison of <u>Rhynchostegium</u> sites by ordination

4.51 The dataset

Environmental characteristics of sites sampled during the intensive survey were summarized using an ordination. Prior to analysis variables were selected which could usefully characterize differences among

Table 4.14 Comparison of skewness (gl) and kurtosis (g2) coefficients for measurements of metals in tips and whole plants of Rhynchostegium, expressed as scalar (untransformed) values and both square root and $\log_{10}$ transformations (* = the form of the variable used for subsequent analyses).

	scalar		square root		$\log_{10}$		
variable	gl	g2	gl	g2	gl	g2	
Na tips	0.44	-0.45	0.09	-0.56	-0.29	-0.32	*
whole	0.47	-0.48	0.21	-0.72	-0.06	-0.78	*
Mg tips	0.76	-0.02	0.48	-0.26	0.16	-0.18	*
whole	0.81	0.25	0.47	-0.02	0.05	0.30	*
K tips	0.63	0.06	0.25	-0.33	-0.13	-0.34	*
whole	0.14	-0.51	-0.19	-0.40	-0.57	0.10	*
Ca tips	1.72	5.63	0.69	3.05	-0.45	3.17	*
whole	1.51	4.81	0.46	2.16	-0.69	2.90	*
Cr tips	7.90	66.8	5.57	34.9	2.73	9.12	*
whole	8.72	79.8	6.04	41.9	2.85	9.77	*
Mn tips	1.94	5.75	0.69	0.01	-0.31	-0.77	*
whole	3.46	17.4	1.11	2.10	-0.51	-0.39	*
Fe tips	4.10	19.4	2.30	7.57	0.42	1.31	*
whole	4.24	21.9	2.42	7.64	0.73	1.20	*
Co tips	2.24	6.53	1.06	1.25	0.04	-0.38	*
whole	2.06	4.39	1.06	0.46	0.15	-0.97	*
Ni tips	1.85	4.62	1.01	1.30	0.30	-0.19	*
whole	3.76	18.0	2.09	5.42	0.82	0.50	*
Cu tips	3.07	11.8	1.74	3.78	0.72	0.23	*
whole	2.60	7.60	1.59	2.68	0.67	0.10	*
Zn tips	2.17	5.24	1.11	0.84	-0.01	-0.35	*
whole	2.50	8.03	1.15	1.40	-0.15	-0.15	*
Cd tips	2.85	12.2	1.15	1.82	-0.14	-0.04	*
whole	1.73	3.78	0.59	0.32	-0.76	0.95	*
Ba tips	2.74	7.45	1.81	2.99	0.80	0.19	*
whole	1.41	1.27	0.81	-0.11	0.10	-0.56	*
Pb tips	4.10	21.9	1.75	4.01	0.10	-0.75	*
whole	3.42	15.5	1.42	2.29	0.02	-0.88	*

sites. All physicochemical variables listed in Table 4.04 were included with the exception of conductivity and optical density. The former is a composite character resulting from other variables already measured (e.g. sulphate, chloride, calcium), which would give excessive weight to these components. The latter were omitted as they were shown to be ambiguous estimates of humic acids (section 4.22), and hence not useful as discriminators between sites.

All aqueous metals analysed were included excepting chromium, for which there was an insufficient number of detectable concentrations. Finally, concentrations of zinc, cadmium, barium and lead in tips were included as estimates of average or "integrated" (Whitton et al., 1981b) heavy metals at each site, in addition to water samples from one point in time.

4.52 Effectiveness of ordination

As ordination may be used for both summarization and prediction, several types of analysis were performed (Table 4.15) to determine the most effective for these data. Criteria included (1) efficiency (the percent of the total variation in the data described by each axis), (2) the dispersion or scatter of cases (sites) along the axes and (3) the amount of information (variables per component) described by each axis. The third criterion is partly subjective in that one's aims may be involved. However, this also depends on whether variables included within a given axis are independent, so that subsequent axes describe other variables and hence are not redundant. These axes are said to be perpendicular, or "orthogonal," in multivariate space. The approach which most closely satsified these requirements was the second, a PCA

Table 4.15. Ordinations tested for summarizing Rhychostegium sites using environmental data (PCA = principal components analysis, FAC = factor analysis, stand = standardized, NA = not applicable; * = method used, see Fig. 4.08).

method	data form	(dis)similarity	stand	rotation	total variance in 1st 3 axes	comments
PCA	scalar	correlation	-	NA	57%	weak scatter, axes orthogonal
PCA	normal	correlation	-	NA	60%	wide scatter, axes othogonal
PCA	normal	covariance	-	NA	96%	no scatter
PCA	normal	correlation	+	NA	59%	wide scatter, axes partly correlated
PCA	normal	covariance	+	NA	59%	wide scatter, axes orthogonal
FAC	normal	least squares	-	varimax	42%	moderate scatter, axes partly correlated
FAC	normal	least squares	-	orthogonal	34%	wide scatter, axes orthogonal

based on the correlation matrix of unstandardized variables. PCA using a covariance matrix resulted in a distortion of the data, failing to produce any dispersion of points unless the variables were standardized first. Factor analysis produced clear scatters but was not as efficient in summarizing the data.

4.53 Empirical results of PCA

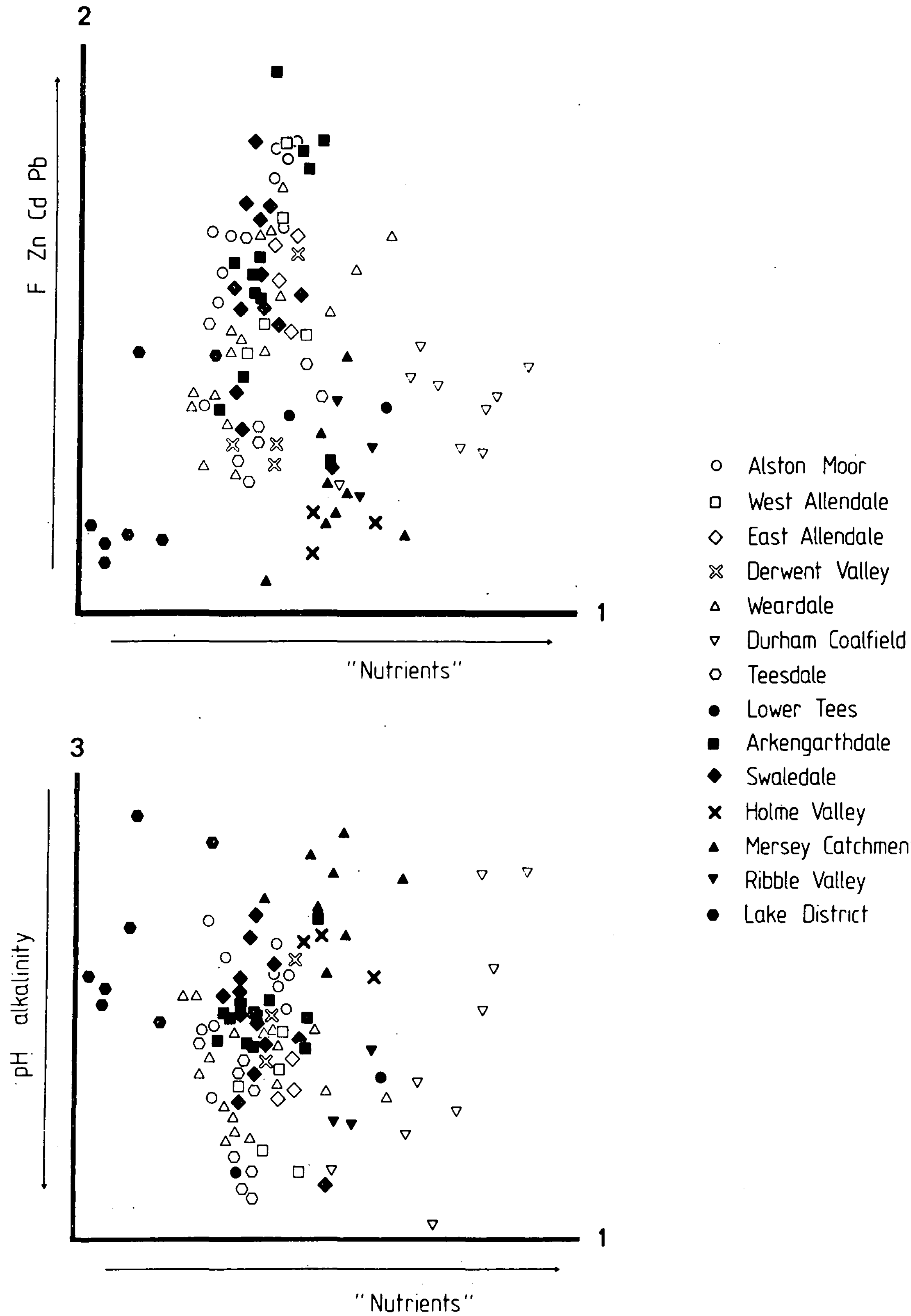
A significance test for the independence between variables produced a χ^2 = 1460 (df = 350), over the first three axes, which is highly significant ($p \ll 0.001$). The solution achieved by this procedure resulted in loadings for the 105 cases (stream and river sites) which were correlated against the original variables (Table 4.16). Many variables were significantly correlated with one or more of the first three component axes, but to different degrees. The principal factors associated with axis one were: sulphate, sodium, magnesium, potassium and chloride in water, all positively. A less important group (also positively correlated) included aqueous nitrite, nitrate and filtrable reactive phosphate (FRP). Axis two was principally associated with heavy metals in water (zinc, cadmium and lead), plus fluoride. Zinc, cadmium and lead (but not barium) in mosses were also strongly related. The third axis was strongly correlated (negatively) with total alkalinity and pH. Some variables, particularly aqueous calcium and silica were less important (relative to the loadings), although correlated to one or more axis.

A geometric representation of the analysis (Fig. 4.08) indicated several relationships between sites. The plot of sites along the first two components indicates a clumped distribution relative to differences in the common anions plus potassium and sodium. As a rough scale of

Table 4.16. Correlation between environmental variables and loadings of sites on the first three principal component axes, also giving the percentage of the total variation expressed in each component (all variables except pH & % O2 saturation were transformed to normal * = p < 0.05, ** = p < 0.01, *** = p < 0.001).

variable	axis 1	axis 2	axis 3
pH	0.31 **	0.42 ***	-0.62 ***
alk	0.56 ***	0.49 ***	-0.56 ***
NH4	0.60 ***	-0.36 ***	0.49 ***
NO2	0.73 ***	-0.41 ***	0.14
NO3	0.76 ***	-0.21 *	0.31 **
FRP	0.65 ***	-0.43 ***	0.21 *
FOP	0.61 ***	-0.29 **	0.21 *
F	0.32 ***	0.59 ***	-0.22 *
Si	0.68 ***	-0.02	0.15
SO4	0.88 ***	0.14	-0.02
Cl	0.83 ***	-0.24 *	0.04
% sat. O2	-0.24 *	0.04	-0.36 ***
Na.wat	0.88 ***	-0.22 *	0.08
Mg.wat	0.87 ***	0.12	-0.22
K.wat	0.87 ***	0.13	-0.08
Ca.wat	0.74 ***	0.44 ***	-0.44 ***
Mn.wat	0.72 ***	-0.10	0.26 **
Fe.wat	0.19 *	-0.10	0.02
Co.wat	0.67 ***	0.20	-0.23 *
Ni.wat	0.79 ***	0.19 *	-0.02
Cu.wat	0.60 ***	0.10	0.27 **
Zn.wat	0.31 **	0.72 ***	0.30 **
Cd.wat	0.07	0.73 ***	0.40 ***
Ba.wat	0.34 ***	0.54 ***	-0.31 ***
Pb.wat	-0.18	0.68 ***	0.18
Zn.tip	0.04	0.67 ***	0.48 ***
Cd.tip	-0.11	0.63 ***	0.58 ***
Ba.tip	-0.22 *	0.31 **	0.14
Pb.tip	-0.26 **	0.77 ***	0.32 ***
% variance			
individual	34.4	17.9	9.9
cumulative	34.4	52.3	62.2

Figure 4.08. Principal components analysis of Rhynchostegium sites showing hydrogeological regions (axes 1 & 2, 1 & 3 are plotted).


2
F Zn Cd Pb
1
"Nutrients"
3
pH alkalinity
1
"Nutrients"
Alston Moor
West Allendale
East Allendale
Derwent Valley
Weardale
Durham Coalfield
Teesdale
Lower Tees
Arkengarthdale
Swaledale
Holme Valley
Mersey Catchment
Ribble Valley
Lake District

increasing "nutrients," the majority of the sites plotted midway along the axis with a few extremes on either end. Those points loading positively were a cluster of sites from lowland Durham. The negative positions were occupied by nutrient-poor sites from the Lake District.

The second principal component, a gradient of heavy metals plus fluoride, exposed an even spread of sites. Lower positions were again represented by several Lake District streams, along with a few upland sites on the River Etherow and the River Holme. The positive end consisted of several sites in Alston Moor, Swaledale and Arkengarthdale. Although the River Team was found to have elevated zinc, these sites (symbols to the right) did not plot as strongly as many streams in mining regions, as concentrations of heavy metals other than zinc were comparatively low.

Axis two also exposed differences within a region. Three of the four Derwent valley sites were in a similar low position. The fourth plotted further up the gradient; a site (0061-08) below the entry of Bolts Burn, with higher heavy metal concentrations (Zn, Cd, Pb: 5 - 10 times greater) and fluoride (three times). Weardale sites plotted over a wide heavy metal range, but most Teesdale sites had lower loadings.

Several regions were widely spread along the third principal component, a gradient of total alkalinity and pH. The comparatively nutrient-rich sites in Durham Coalfield were some of the least and most strongly buffered and alkaline waters where <u>Rhynchostegium</u> was found. Some River Team sites, although high in magnesium and calcium (Appendix 2), plotted with sites of lower alkalinity; a site on the Deerness, (also Ca/Mg-rich) plotted more stongly than any other along this gradient. Several upland sites (mostly nutrient-poor) in Weardale, West Allendale and Teesdale actually plotted more strongly (= lower) along the alkalinity gradient than did some nutrient-rich River Team sites.

Gaps in the geometric plot of sites reveal the types of streams and rivers which were either not sampled or are environments which Rhynchostegium did not occupy. Two obvious spaces exist in the plot of components one v. two. The upper left hand space would be streams with low concentrations of sulphate, sodium, magnesium, potassium, chloride and probably nitrate and phosphate as well (comparable to the Lake District), but "high" concentrations of heavy metals (e.g. > 1.0 mg l^{-1} Zn). Similar streams were visited during preliminary surveys (e.g. small flushes from mine tailings), but were usually barren or colonized by Scapania undulata. The second apparent gap plotted in the upper right of the plot would be sites of high heavy metals and high "nutrients" (e.g. > 50 mg l^{-1} SO_4-S and 5 mg l^{-1} NO_3-N). None of these sites were encountered during the intensive survey (but see seasonal surveys, chapter 5).

4.6 Analysis of factors affecting metal accumulation by field populations of Rhynchostegium

4.61 Bivariate results

The first stage in examining metal accumulation by in situ populations was the simple relationship between concentrations of metals in stream water and in mosses. Correlations were performed initially using both total and filtrable aqueous metals, with metals in both 2 cm apical tips and whole plants of Rhynchostegium (Table 4.17). Nearly all metals were significantly correlated, with the exception of iron, cobalt and nickel. Of the remaining 10 elements only calcium correlated more strongly (marginally) with whole plants. All other elements correlated more strongly with tips than whole plants, or were similar between the two. Correlations for total and filtrable metals were similar, with the exception of barium, where a marked improvement in the correlation was attained with the filtrable fraction.

A second bivariate relation commonly used to describe accumulation is the enrichment ratio, is defined as the concentration of a metal in an organism divided by the concentration in water (assuming equivalent units, e.g. $\mu g\ g^{-1}$ dry weight and $mg\ l^{-1}$: both = parts per million). This ratio was calculated for seven metals (Co, Ni, Cu, Zn, Cd, Ba, Pb) in tips and whole plants of Rhynchostegium (Table 4.18). Results indicate that amongst the populations sampled, this ratio for any one element varied widely. Coefficients of variation ranged from about 70% (copper in tips and whole plants) to as much as 200% (lead in tips). Ratios for each metal were on average higher in whole plants, but due

Table 4.17. Correlations between concentrations of aqueous and accumulated metals for 105 populations of Rhynchostegium, comparing 2 cm apical tips and whole plants (all data are normalized via $\log_{10}$ transformation; blanks indicate insufficient data; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

element		apical tips	whole plants
Na	T	0.37 ***	0.20 *
	F	0.37 ***	0.20 *
Mg	T	0.42 ***	0.34 ***
	F	0.42 ***	0.34 ***
K	T	0.51 ***	0.47 ***
	F	0.50 ***	0.47 ***
Ca	T	0.46 ***	0.53 ***
	F	0.46 ***	0.53 ***
Cr	T		
	F		
Mn	T	0.34 ***	0.32 ***
	F	0.33 ***	0.31 ***
Fe	T	0.13	0.18
	F	0.04	0.14
Co	T	0.04	0.13
	F		
Ni	T	0.17	0.15
	F		
Cu	T	0.42 ***	0.40 ***
	F		
Zn	T	0.81 ***	0.68 ***
	F	0.80 ***	0.66 ***
Cd	T	0.65 ***	0.44 ***
	F	0.65 ***	0.44 ***
Ba	T	0.27 **	0.17
	F	0.48 ***	0.38 ***
Pb	T	0.75 ***	0.74 ***
	F	0.70 *	0.69 *

Table 4.18. Enrichment ratios for cobalt, nickel, copper, zinc, cadmium, barium and lead in apical tips and whole plants (n = 105) of Rhynchostegium from intensive stream survey.

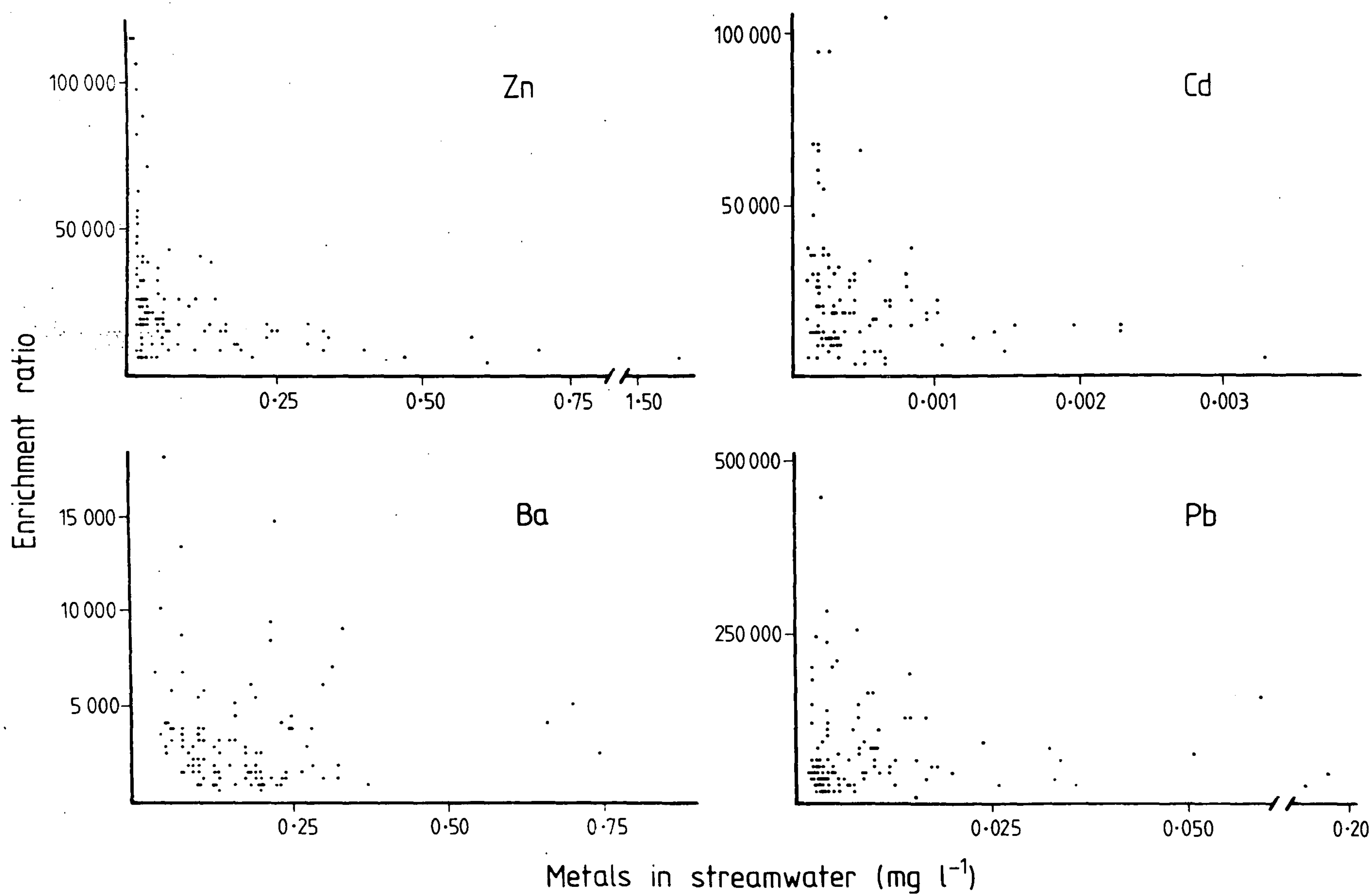
element fraction		minimum	maximum	mean	standard deviation
Co	tips	130	34200	3810	4600
	whole	880	165000	17600	25400
Ni	tips	430	15100	2440	3550
	whole	626	72500	6620	9720
Cu	tips	643	24800	4680	3550
	whole	824	34500	7180	5230
Zn	tips	3820	183000	29400	35500
	whole	5760	538000	84900	118000
Cd	tips	2170	104000	23400	19500
	whole	1240	340000	64700	60400
Ba	tips	574	27000	3480	3790
	whole	1050	46000	7400	7190
Pb	tips	1010	2510000	94400	248000
	whole	2200	3860000	217000	417000

to the extreme variation, these differences are statistically insignificant. Between metals, the highest ratios were for lead. Relationships between enrichment ratios and metals in stream water were not constant for any element (e.g. Zn, Cd, Ba, Pb: Fig. 4.09). This shows that as the concentration of an aqueous metal increases, the ratio decreases. Further, this relation was nonlinear.

4.62 Multivariate results

Multiple stepwise regressions were used to examine what factors may have affected heavy metal accumulation by field populations of Rhynchostegium in situ. The metals cobalt, nickel, copper, zinc, cadmium, barium and lead were studied separately. Results for apical tips and whole plants are presented in order to compare any differences which may exist in their responses to environmental factors. For each a simple scatter of the bivariate regression of aqueous metal v. accumulated metal precedes the new regression line. The second line is

Figure 4.09. Scattergrams showing relationship between enrichment ratios in Rhynchostegium and aqueous metals for Zn, Cd, Ba, Pb.



Zn
Cd
Ba
Pb
100 000
50 000
15 000
10 000
5 000
500 000
250 000
0·25
0·50
0·75
1·50
0·001
0·002
0·003
0·025
0·050
0·20
Enrichment ratio
Metals in streamwater (mg l⁻¹)

based on the concentrations predicted by multivariate regression, taking into account those significant factors (mimimum acceptable = $p < 0.05$) included in the analysis.

4.621 Cobalt

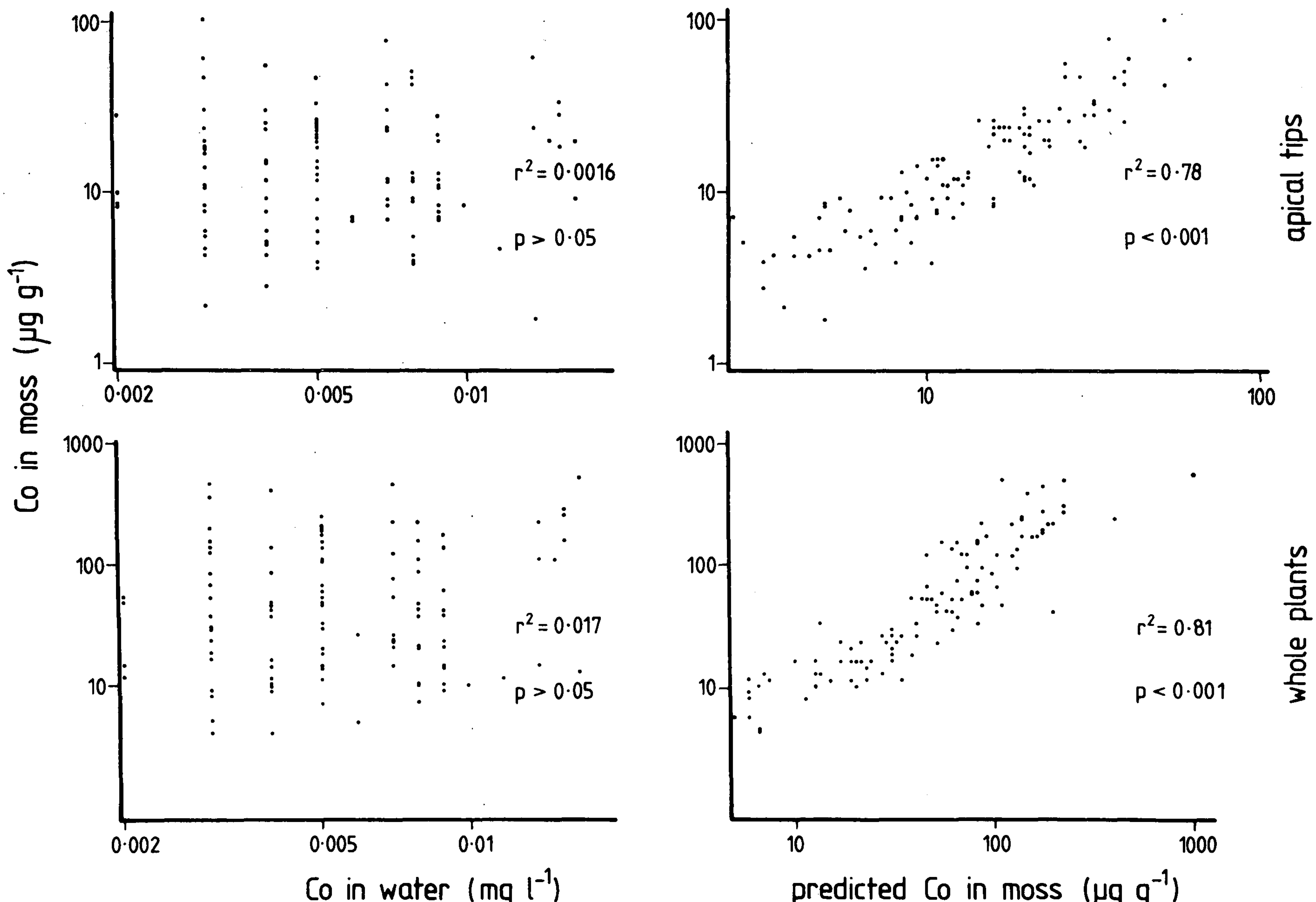
Multiple regression indicated that six factors were significantly correlated with cobalt accumulation in tips and three factors with cobalt in whole plants (Table 4.19). For neither fraction was aqueous

Table 4.19. Variables extracted in multiple stepwise regression, using Co in tips and whole plants as dependent variables (+/- = sign of effect; SE = standard error; p = significance (* = p < 0.05, ** = p < 0.01, *** = p < 0.001) of each step).

	Co: tips					Co: whole plants				
step	var	+/-	slope	SE	p	var	+/-	slope	SE	p
1	Mn.tip	+	0.54	0.04	***	Mn.whp	+	0.75	0.04	***
2	Fe.tip	+	0.30	0.06	***	Fe.whp	+	0.38	0.08	***
3	Zn.wat	+	0.16	0.04	**	Zn.wat	+	0.14	0.04	**
4	TA	-	0.16	0.06	**					
5	Ca.tip	+	0.35	0.15	*					
6	Mn.wat	+	0.13	0.04	**					
	constant	-	0.61	0.13	***	constant	-	1.24	0.17	***

cobalt indicated to be an important factor, however. Manganese and iron in tips and whole plants were the first factors selected. Aqueous zinc also correlated positively. Concentrations in tips were apparently affected by several other factors, although their signficance was lower. Bivariate scatters for this metal in stream water and plants (Fig. 4.10) were not significantly correlated, but the predicted v. accumulated metals for both plant fractions had a fairly good fit. These scatters and the table of factors indicated by multiple

Figure 4.10. Actual and predicted regressions for accumulation of Co by apical tips and whole plants of Rhynchostegium.


Co in moss (μg g⁻¹)
100
10
1
0·002
0·005
0·01
r² = 0·0016
p > 0·05
100
10
1
10
100
r² = 0·78
p < 0·001
apical tips
1000
100
10
0·002
0·005
0·01
r² = 0·017
p > 0·05
Co in water (mg l⁻¹)
1000
100
10
10
100
1000
r² = 0·81
p < 0·001
whole plants
predicted Co in moss (μg g⁻¹)

regression suggest that aqueous cobalt was not a significant factor contributing to greater cobalt accumulation by mosses in the streams sampled.

4.622 Nickel

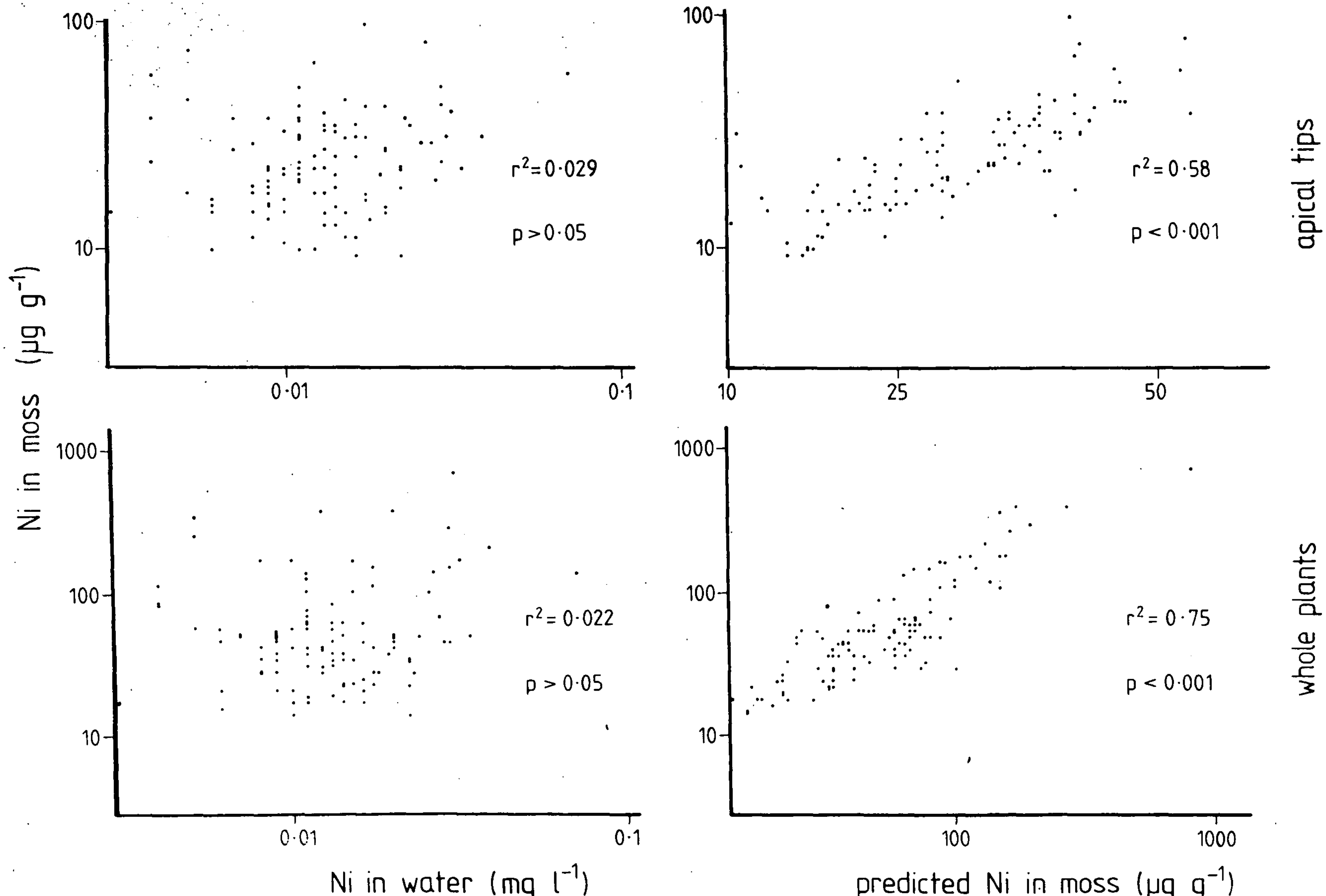
Eight factors contributed significantly to the regression model for nickel in tips, while ten were selected using nickel in whole plants as the dependent variable (Table 4.20). Variables which were selected

Table 4.20. Variables extracted in multiple stepwise regression, using Ni in tips and whole plants as dependent variables (+/- = sign of effect; SE = standard error; p = significance (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$) of each step).

	Ni: tips					Ni: whole plants				
step	var	+/-	slope	SE	p	var	+/-	slope	SE	p
1	K.wat	+	0.21	0.04	***	Mn.whp	+	0.35	0.05	***
2	Mn.tip	+	0.11	0.04	**	OD.240	-	0.69	0.13	***
3	Zn.wat	+	0.10	0.04	**	K.wat	+	0.44	0.10	**
4	TA	-	0.22	0.08	**	pH	-	0.18	0.05	**
5	Ca.tip	+	0.39	0.14	**	FRP	-	0.10	0.04	**
6	OD.240	-	0.32	0.09	***	Cu.wat	+	0.22	0.08	*
7	Si	+	0.18	0.08	**	% O2	+	0.02	0.01	*
8	FRP	-	0.07	0.03	*	Ca.whp	+	0.31	0.15	*
9						Si	+	0.24	0.12	*
10						Ca.wat	-	0.27	0.13	*
	constant		1.36	0.02	***	constant		0.36	0.18	***

were somewhat similar for the two plant fractions, although the order of their importance differs. Of these, aqueous potassium and manganese in the moss were positively related. Aqueous nickel was also not included in either regression. The scatter diagrams (Fig. 4.11) show clearly the weak relation between aqueous and accumulated nickel. The predicted regression was again closer for whole plants than for tips.

Figure 4.11. Actual and predicted regressions for accumulation of Ni by apical tips and whole plants of Rhynchostegium.



Ni in moss (µg g^{-1})
$r^2 = 0.029$
$p > 0.05$
$r^2 = 0.58$
$p < 0.001$
apical tips
$r^2 = 0.022$
$p > 0.05$
$r^2 = 0.75$
$p < 0.001$
whole plants
Ni in water (mg l^{-1})
predicted Ni in moss (µg g^{-1})

4.623 Copper

This metal differs from the previous two in that the multiple regression selected the aqueous metal as one factor significantly related to accumulation in the moss (Table 4.21). There was also a

Table 4.21. Variables extracted in multiple stepwise regression, using Cu in tips and whole plants as dependent variables (+/- = sign of effect; SE = standard error; p = significance (* = p < 0.05, ** = p < 0.01, *** = p < 0.001) of each step).

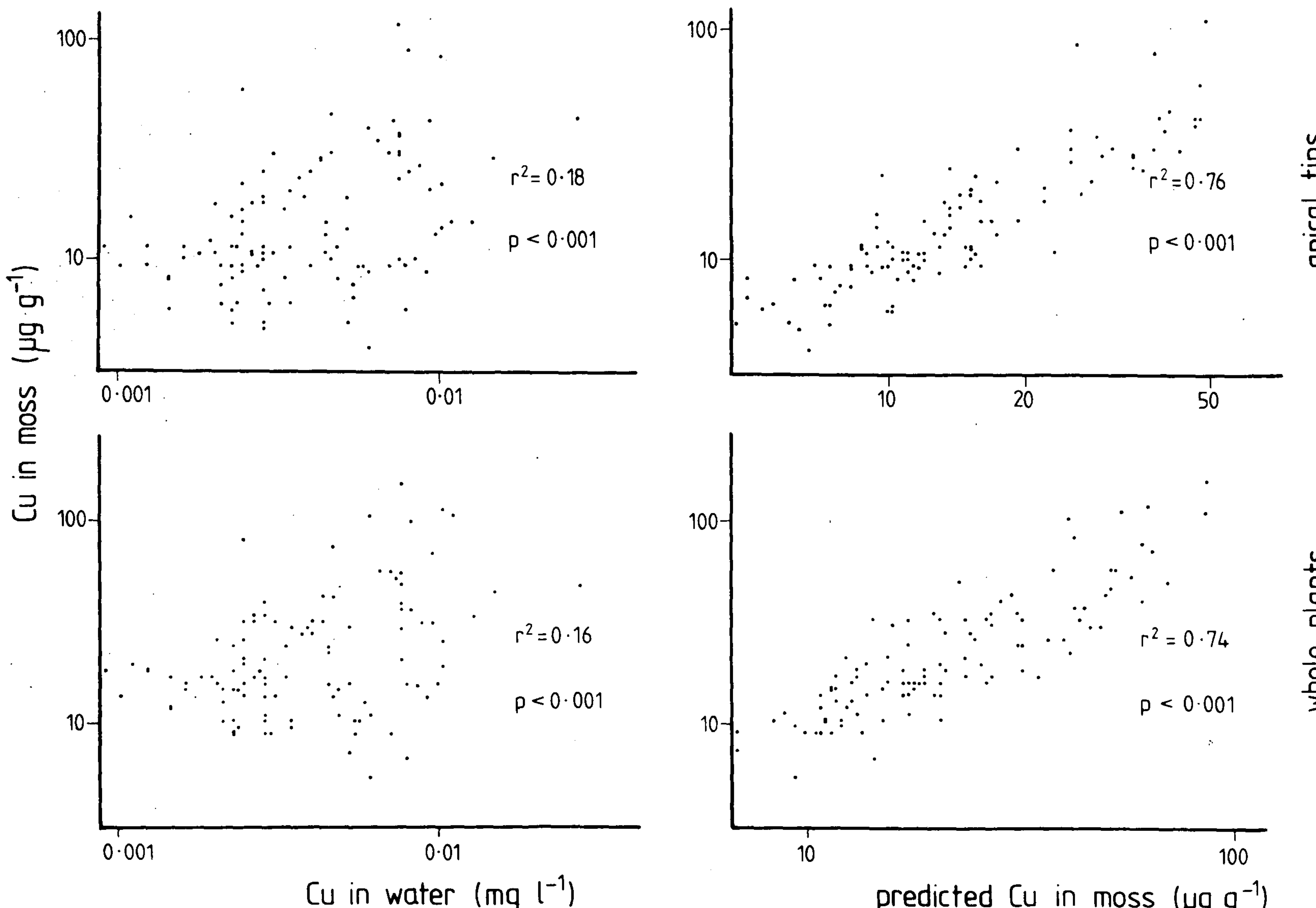
	Cu: tips					Cu: whole plants				
step	var	+/-	slope	SE	p	var	+/-	slope	SE	p
1	Mn.wat	+	0.33	0.04	***	Mn.wat	+	0.35	0.04	***
2	Mg.tip	+	1.02	0.20	***	OD.240	-	0.49	0.11	***
3	Cu.wat	+	0.25	0.08	**	Cu.wat	+	0.30	0.08	***
4	Mg.wat	-	0.24	0.07	**	Fe.whp	+	0.16	0.07	*
5	OD.240	-	0.49	0.13	***	Na.whp	+	0.37	0.15	*
6	Fe.tip	+	0.19	0.06	**	Si	-	0.25	0.10	*
7	SO4	+	0.44	0.11	***	Mg.whp	+	0.38	0.17	*
8	Ca.wat	+	0.41	0.14	**	Mg.wat	-	0.17	0.08	*
9	temp	+	0.02	0.01	*	SO4	+	0.39	0.11	***
	constant	+	1.71	0.08	***	constant	+	1.93	0.08	***

greater similarity in the complete group of variables for the two plant fractions, with aqueous manganese positively and magnesium, negatively related. Optical density was also selected (negatively). The two bivariate regressions with aqueous copper, although weak, were significant, unlike those for cobalt and nickel (Fig. 4.12). Fitted lines resulting from the multiple regression were similar for the two plant fractions.

4.624 Zinc

Unlike the previous elements, simple bivariate regressions for zinc accumulation with aqueous zinc fitted a straight line fairly well (Fig.

Figure 4.12. Actual and predicted regressions for accumulation of Cu by apical tips and whole plants of Rhynchostegium.



Cu in moss (μg g⁻¹)
100
10
0·001
0·01
Cu in water (mg l⁻¹)
r² = 0·18
p < 0·001
r² = 0·16
p < 0·001
10
20
50
100
predicted Cu in moss (μg g⁻¹)
r² = 0·76
p < 0·001
r² = 0·74
p < 0·001
apical tips
whole plants

4.13). Further, aqueous zinc was first selected by multiple regression (Table 4.22). The complete list of variables differ between tips and

Table 4.22. Variables extracted in multiple stepwise regression, using Zn in tips and whole plants as dependent variables (+/- = sign of effect; SE = standard error; p = significance (* = p < 0.05, ** = p < 0.01, *** = p < 0.001) of each step).

	Zn: tips					Zn: whole plants				
step	var	+/-	slope	SE	p	var	+/-	slope	SE	p
1	Zn.wat	+	0.60	0.04	***	Zn.wat	+	0.48	0.05	***
2	Ca.wat	-	0.46	0.09	***	Mn.whp	+	0.30	0.06	***
3	Ca.tip	+	0.85	0.17	***	FRP	-	0.18	0.04	***
4	Mn.tip	+	0.18	0.04	***	Mg.wat	-	0.32	0.09	***
5	Fe.wat	-	0.14	0.05	**	Cd.wat	+	0.31	0.11	**
6	Fe.tip	+	0.16	0.07	*	Na.wat	+	0.25	0.12	*
7	pH	+	0.13	0.06	*					
8	NH4	+	0.10	0.04	*					
	constant	+	3.75	0.07	***	constant	+	3.96	0.08	***

whole plants. The analysis indicates zinc in tips correlated (after aqueous zinc) principally with the negative factors aqueous calcium and iron and the positive factors calcium and manganese in tips. Whole plants were also correlated positively with accumulated manganese, but negatively with filtrable reactive phosphate and aqueous magnesium. The final predicted regression lines based on these factors, were improved over the simple bivariate scatters, although the initial configurations were nearly linear at the onset.

4.625 Cadmium

Simple regressions for cadmium (Fig. 4.14) were not as strong as for zinc, but the multiple regressions for both tips and whole plants suggest aqueous cadmium was the principal factor affecting accumulation (Table 4.23). Multiple regression included a few more vari-

Figure 4.13. Actual and predicted regressions for accumulation of Zn by apical tips and whole plants of Rhynchostegium.

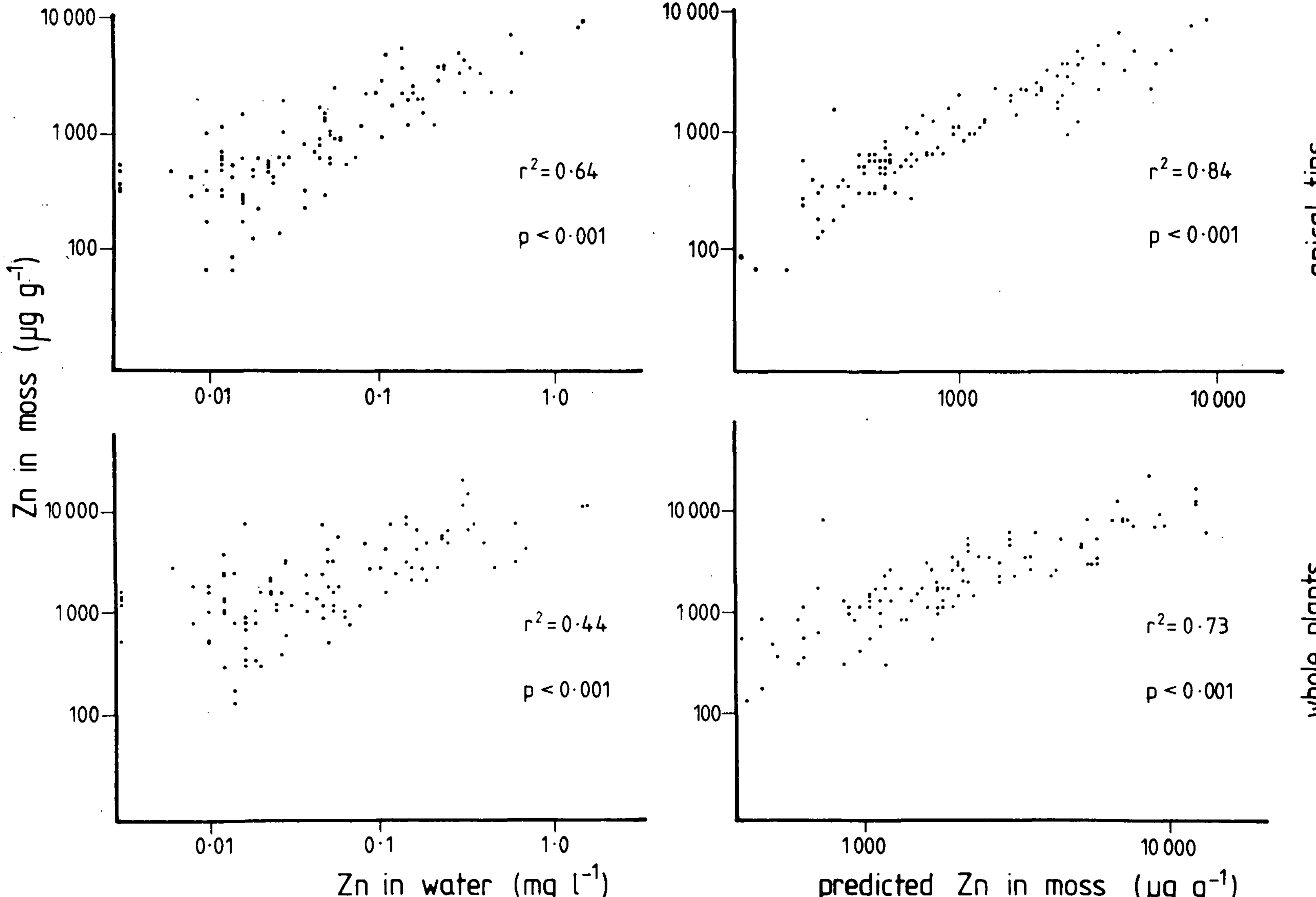

10 000
1 000
100
0·01
0·1
1·0
r^2 = 0·64
p < 0·001
1000
10 000
r^2 = 0·84
p < 0·001
apical tips
Zn in moss (µg g^{-1})
r^2 = 0·44
p < 0·001
r^2 = 0·73
p < 0·001
whole plants
Zn in water (mg l^{-1})
predicted Zn in moss (µg g^{-1})

Figure 4.14. Actual and predicted regressions for accumulation of Cd by apical tips and whole plants of Rhynchostegium.

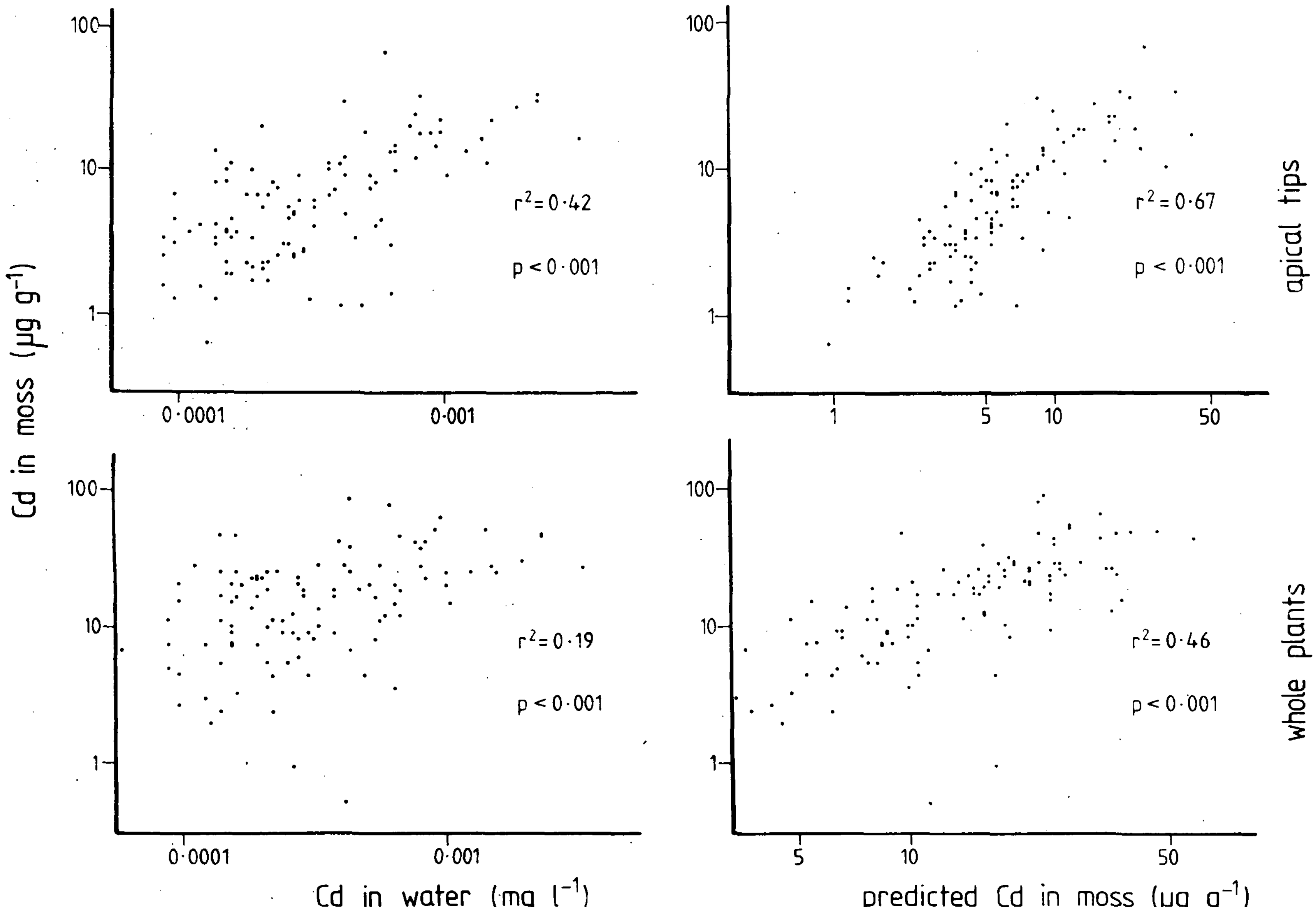

Cd in moss (µg g^{-1})
100
10
1
0·0001
0·001
$r^2 = 0·42$
$p < 0·001$
1
5
10
50
$r^2 = 0·67$
$p < 0·001$
apical tips
$r^2 = 0·19$
$p < 0·001$
5
10
50
$r^2 = 0·46$
$p < 0·001$
whole plants
Cd in water (mg l^{-1})
predicted Cd in moss (µg g^{-1})

Table 4.23. Variables extracted in multiple stepwise regression, using Cd in tips and whole plants as dependent variables (+/- = sign of effect; SE = standard error; p = significance (* = p < 0.05, ** = p < 0.01, *** = p < 0.001) of each step).

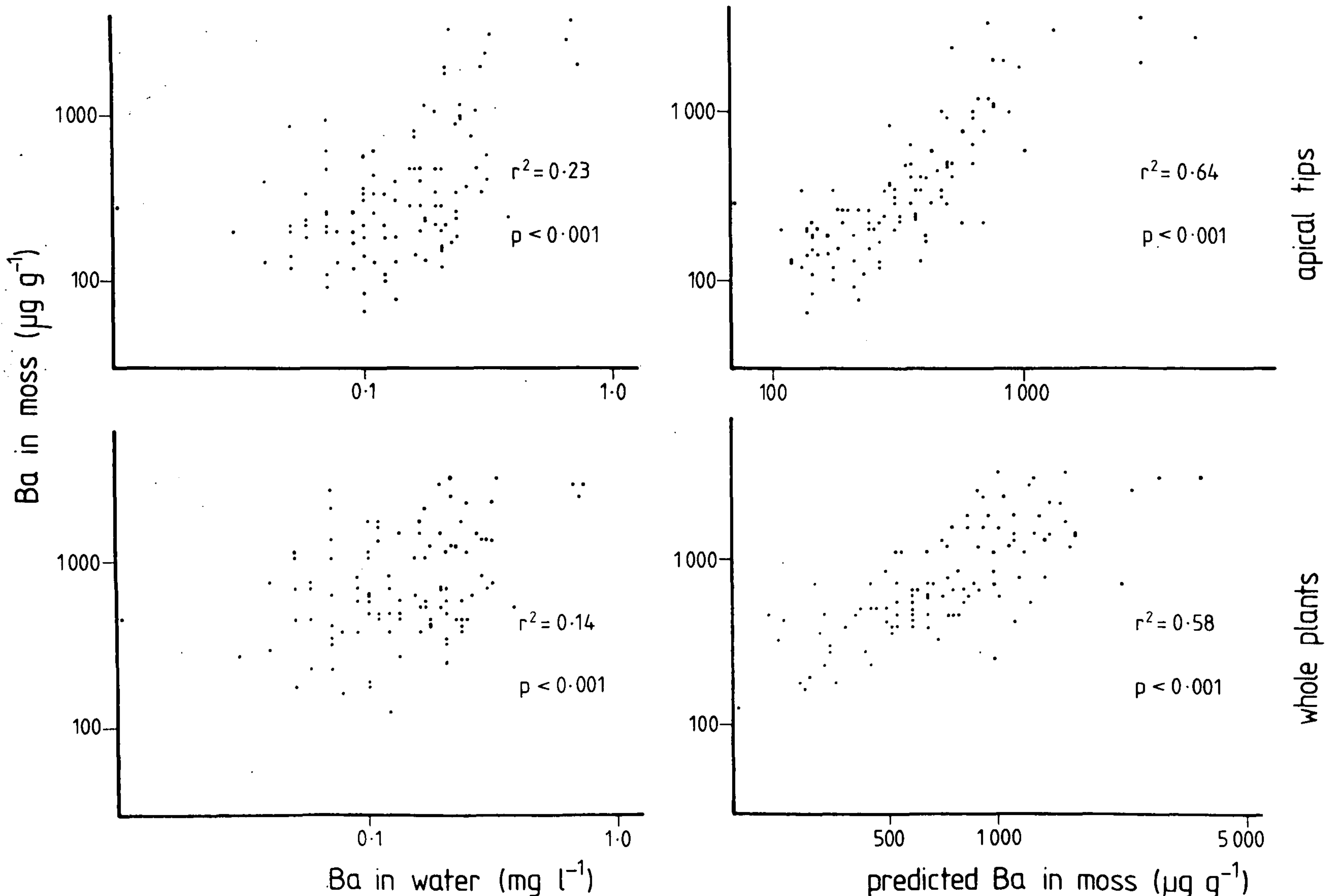
	Cd: tips					Cd: whole plants				
step	var	+/-	slope	SE	p	var	+/-	slope	SE	p
1	Cd.wat	+	0.76	0.09	***	Cd.wat	+	0.49	0.10	***
2	Fe.tip	+	0.31	0.10	***	K.whp	-	1.03	0.25	***
3	Pb.wat	+	0.26	0.07	***	Mn.whp	+	0.23	0.06	***
4	Mn.wat	-	0.17	0.06	**	TA	-	0.26	0.10	**
5	NO3	+	0.14	0.05	**	Pb.wat	+	0.21	0.08	**
6	Mn.tip	+	0.21	0.07	**					
7	Fe.wat	-	0.16	0.06	*					
	constant	+	3.40	0.30	***	constant	+	2.87	0.35	***

ables in the model for tips than for whole plants, as was found for zinc. Amongst these, iron and manganese in tips and aqueous lead and nitrate were positively related, while iron and manganese in water were negatively related to cadmium in tips. Concentrations of potassium in whole plants and total alkalinity are factors that may have negatively influenced accumulation by whole plants. Manganese in plants and aqueous lead were again related positively.

4.626 Barium

Aqueous barium was significantly correlated with both plant fractions (Fig. 4.15), but weakly, compared to zinc, cadmium and lead (see section 4.627). Multiple regressions for barium nonetheless indicated the aqueous metal was the factor most significantly correlated with concentrations in both plant fractions (Table 4.24). Other factors selected by the analysis were similar for the two fractions. Aqueous

Figure 4.15. Actual and predicted regressions for accumulation of Ba by apical tips and whole plants of Rhynchostegium.



Ba in moss (μg g⁻¹)
1000
100
0·1
1·0
r² = 0·23
p < 0·001
1 000
100
100
1 000
r² = 0·64
p < 0·001
apical tips
1000
100
0·1
1·0
r² = 0·14
p < 0·001
1 000
100
500
1 000
5 000
r² = 0·58
p < 0·001
whole plants
Ba in water (mg l⁻¹)
predicted Ba in moss (μg g⁻¹)

Table 4.24. Variables extracted in multiple stepwise regression, using Ba in tips and whole plants as dependent variables (+/- = sign of effect; SE = standard error; p = significance (* = p < 0.05, ** = p < 0.01, *** = p < 0.001) of each step).

	Ba: tips					Ba: whole plants				
step	var	+/-	slope	SE	p	var	+/-	slope	SE	p
1	Ba.wat	+	0.64	0.11	***	Ba.wat	+	0.42	0.10	***
2	Ca.wat	-	0.93	0.11	***	Ca.wat	-	0.59	0.11	***
3	Ca.tip	+	0.52	0.21	*	Mn.whp	+	0.22	0.04	***
4	NO3	+	0.11	0.04	*	Ca.whp	+	0.69	0.16	***
5	Pb.wat	+	0.13	0.06	*	Mg.whp	+	0.40	0.21	*
6	Mn.tip	+	0.12	0.06	*	K.whp	-	0.37	0.18	*
7	Na.wat	-	0.28	0.13	*					
	constant	+	3.07	0.10	***	constant	+	3.22	0.17	***

calcium was the second factor in each and was negatively related. Those positively correlated included calcium and manganese in the plants.

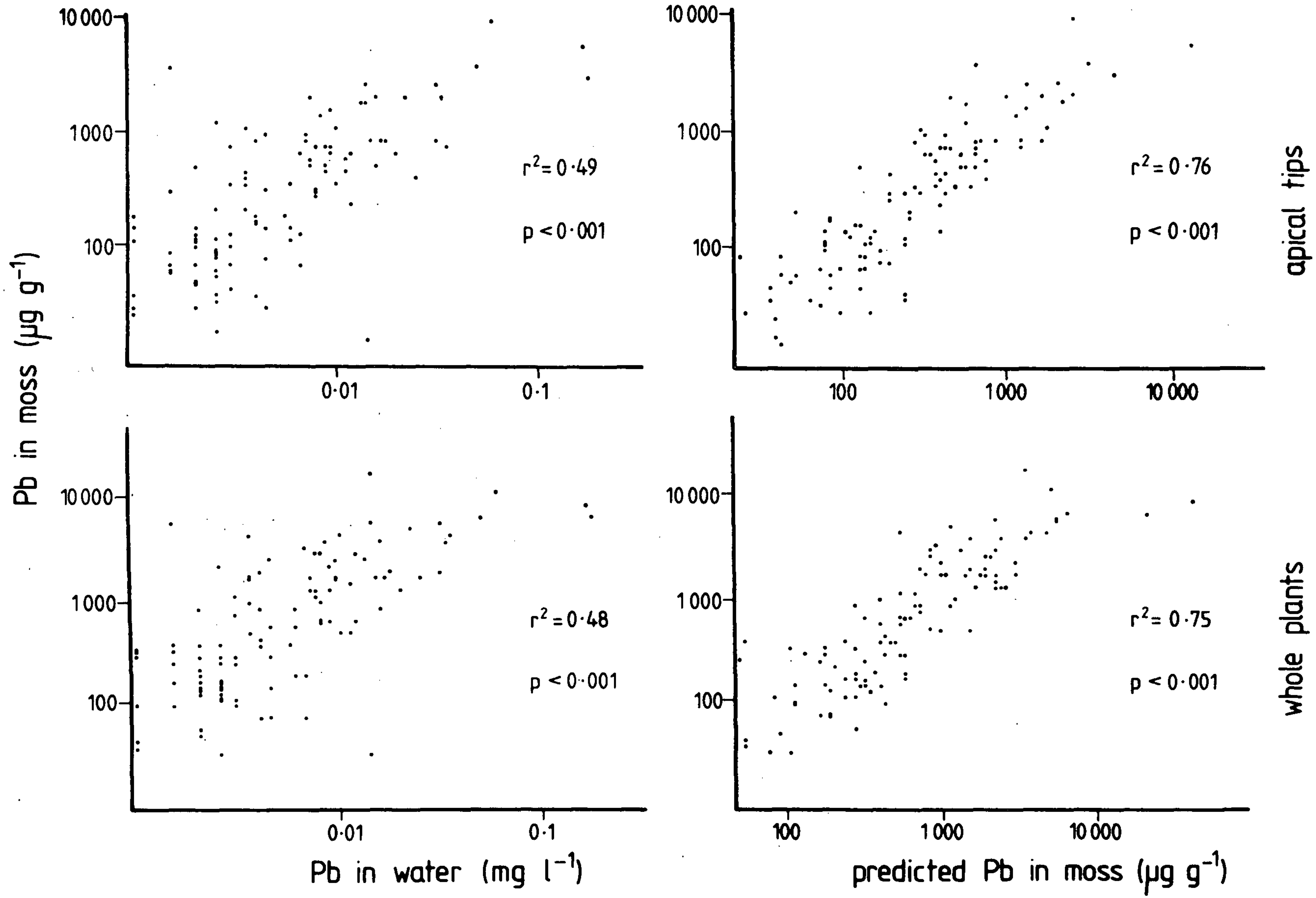
4.627 Lead

Strong positive regressions were found between aqueous and accumulated lead (Fig. 4.16). These relations are supported by multiple regression, as this variable was of principal importance in both models (Table 4.25). The second factor for both plant fractions, filtrable

Table 4.25. Variables extracted in multiple stepwise regression, using Pb in tips and whole plants as dependent variables (+/- = sign of effect; SE = standard error; p = significance (* = p < 0.05, ** = p < 0.01, *** = p < 0.001) of each step).

	Pb: tips					Pb: whole plants				
step	var	+/-	slope	SE	p	var	+/-	slope	SE	p
1	Pb.wat	+	0.93	0.09	***	Pb.wat	+	0.96	0.10	***
2	FRP	-	0.25	0.06	***	FRP	-	0.32	0.06	***
3	Zn.wat	+	0.26	0.07	***	NH4	+	0.33	0.10	**
4	NH4	+	0.27	0.10	**	F^-	+	0.28	0.09	**
5	Ca.tip	+	0.84	0.28	**	Mg.whp	-	0.43	0.13	**
6	TA	-	0.42	0.13	**	Zn.wat	+	0.19	0.07	*
7	Fe.tip	+	0.28	0.12	*	Fe.whp	+	0.26	0.11	*
8	Mn.wat	-	0.27	0.09	**					
	constant	+	4.54	0.22	***	constant	+	4.98	0.23	***

Figure 4.16. Actual and predicted regressions for accumulation of Pb by apical tips and whole plants of <u>Rhynchostegium</u>.



10 000
1000
100
0·01
0·1
$r^2 = 0·49$
$p < 0·001$
10 000
1000
100
100
1000
10 000
$r^2 = 0·76$
$p < 0·001$
apical tips
Pb in moss (μg g^{-1})
10 000
1 000
100
0·01
0·1
$r^2 = 0·48$
$p < 0·001$
10 000
1 000
100
100
1 000
10 000
$r^2 = 0·75$
$p < 0·001$
whole plants
Pb in water (mg l^{-1})
predicted Pb in moss (μg g^{-1})

reactive phosphate, was negatively correlated. The complete lists of implied factors differ between tips and whole plants after the second step in the analysis. Common variables include aqueous zinc, ammonia and iron in plants. Lead, like copper, differed from the other five metals in that the regression did not relate manganese in plants to accumulation.

4.628 Synthesis and hypotheses

The results of the field data for metal accumulation by *in situ* populations of *Rhynchostegium* may be summarized as follows:

1) Cobalt and nickel in mosses did not correlate significantly with concentrations in water.

2) Copper, zinc, cadmium, barium and lead in mosses significantly correlated with concentrations of the respective metal in water.

3) Multiple regression models resulted in significant positive regressions for all metals tested (in both plant fractions), but only those aqueous elements given in point (2) were included as significant factors in the models for accumulation of each element.

4) Multiple regression selected lists of variables which were implied as factors significantly correlated (+ or -) with accumulation of metals in the mosses.

Although variables selected by multiple regression differed between metals (Tables 4.19 - 4.25), several recurring factors can be stated. Among the water chemistry variables, calcium (-), magnesium (-), iron (-), manganese (+/-), phosphorus (-), nitrate (+) and ammonia (+) were commonly included. Concentrations of manganese (+), iron (+), calcium (+) and potassium (-) in mosses themselves were another group of factors suggested by the analysis. The effects of some of these factors were examined experimentally in chapter 7.

CHAPTER 5. SEASONAL SURVEY OF METAL ACCUMULATION BY AQUATIC BRYOPHYTES

5.1 Introduction

This chapter presents the results of a seasonal survey of moss populations from seven contrasting streams. Sites were sampled at monthly intervals (except during floods or freezes) between May 1981 and June 1982. Details, photographs and locations of these sites were given in chapter 3 (section 3.3). Rhynchostegium riparioides was sampled from all seven sites, while Amblystegium riparium and Fontinalis antipyretica were each sampled from one site.

Results from four upland and three lowland sites are divided into sections 5.2 and 5.3, respectively. Data on the seasonal variation in the abundance of aquatic bryophytes, water chemistry and metal accumulation are presented for each site separately. The data include a comparison of metal accumulation by the three species of aquatic moss. The effect of seasonal changes in environmental variables on accumulation is examined through correlations with enrichment ratios (section 4.61) monthly. In section 5.4, the results from the seven contrasting sites are compared. In section 5.5 results from a time-series analysis are presented (temporal cross-correlations) using 3 month successive lags, to consider staggered relationships of accumulation (e.g. seasonal differences in the mosses). Results are then summarized in hypotheses (section 5.55) which attempt to explain the seasonal differences observed.

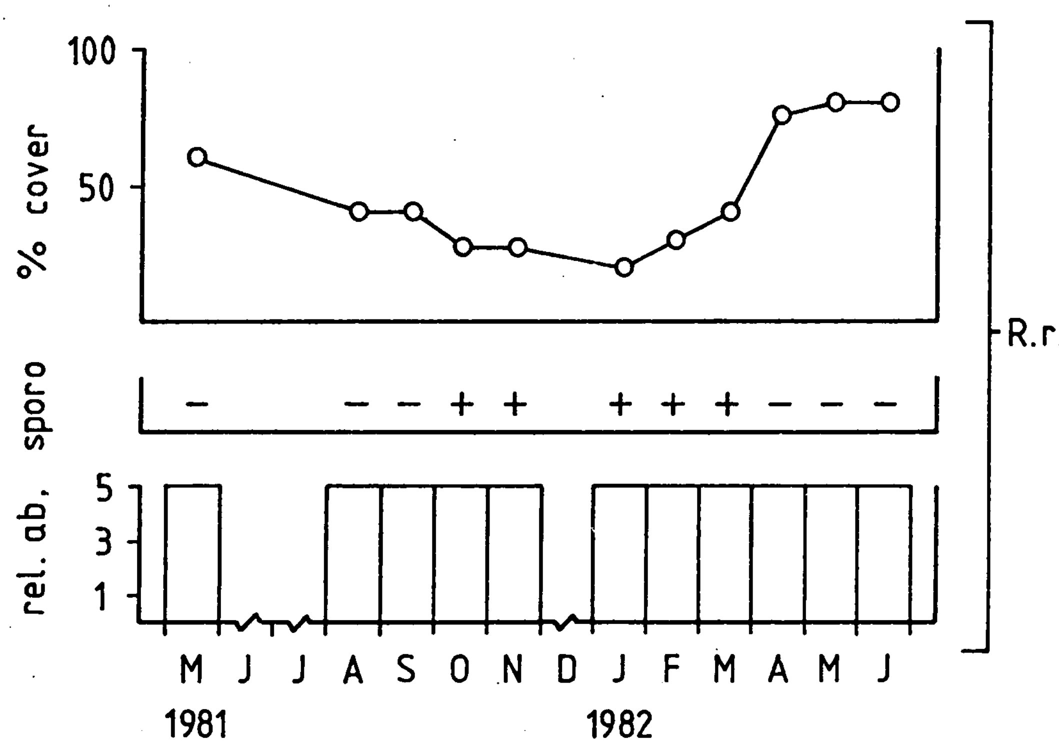
5.2 Descriptive statistics for four upland streams.

Results for four upland streams are presented in order of their hydrogeological region (see section 3.3). Region I, Alston Moor, was represented by "High Crag Burn" (0101-05); region II, West Allendale, by Lee Springs (0289-98) and the River West Allen (0085-50); and region V, Weardale, by "Race Fell Burn" (0310-90). All four sites were sampled during 11 months of the 14 month period. These sites could not be sampled in late spring due to high flows or in December when higher elevation roads were snowed in. Results for this section and the following section are divided into three parts. First, data for the relative abundance of all aquatic bryophytes and macrophytic algae are presented. Results are given for all those species which were present in a submerged habit for two or more months of the year. Cover estimates and the phenology of sporophyte production for Rhynchostegium, are also presented. The second part in each describes the seasonal variation in physicochemical variables and the aqueous metals sodium, magnesium, calcium, manganese and iron. The third part presents results for aqueous and accumulated zinc, cadmium, barium and lead.

5.21 "High Crag Burn" (0101-05)

The only aquatic bryophyte in this site was Rhynchostegium. Not only was it the dominant macrophyte (abundance rank = 5) year round, but also had a high percentage cover (Fig. 5.01). Cover decreased over the autumn-winter period, but by May 1982 the moss had increased to 80% cover of the streambed. Sporophyte production by this population

Figure 5.01. Seasonal changes in the relative abundance (1 - 5) of aquatic bryophytes and macroalgae, and the percent cover and sporophyte production of Rhynchostegium in "High Crag Burn" (R.r. = Rhynchostegium riparioides).


100
50
% cover
sporo
rel. ab.
5
3
1
− − − + + + + + − − −
M J J A S O N D J F M A M J
1981
1982
R.r.

was restricted to October through March, when absolute abundance of plants was at a minimum. These were found on both submerged and emergent plants.

Minimum temperatures (about 3 ^{o}C) and maximum optical densities were measured during winter and early spring (Fig. 5.02). Total alkalinity and pH also dropped, although the stream remained fairly well buffered (1 - 2 meq l^{-1}) at minimum pH values (pH 6.9 - 7.1). Both forms of phosphorus, although low, increased over the autumn-winter period. Chloride and silica had less obvious seasonal patterns. An obvious peak in chloride during February coincided with winter salting of the roads.

Concentrations of several aqueous metals varied similarly over the year (Fig. 5.03). Magnesium, calcium and manganese clearly varied similarly, with maxima during summer. An abrupt reduction in concentrations followed in October (to about 30% of the maxima) which continued through January. Aqueous iron followed a nearly opposite pattern (similar to O.D.) to these metals. Sodium was apparently less seasonal in its variation.

The metals zinc, cadmium, barium and lead in stream water and in <u>Rhynchostegium</u> followed somewhat complicated patterns (Fig. 5.04). Seasonal changes in zinc concentrations in these two components were closely related. Like other metals, such as magnesium and calcium, concentrations of zinc decreased over the autumn-winter period. Maximum aqueous zinc (F = 1.16 mg l^{-1}) was measured in June 1982. Cadmium in stream water varied similarly, but the correspondence with accumulation was less direct. Seasonal variations in aqueous barium and lead were more complex; an increase in barium from May 1981 to January 1981 corresponded with a period of decrease in the moss. Temporal

Figure 5.02. Seasonal changes in temperature, optical density, pH, total alkalinty, phosphate (filtrable organic & reactive), chloride and silica in "High Crag Burn."

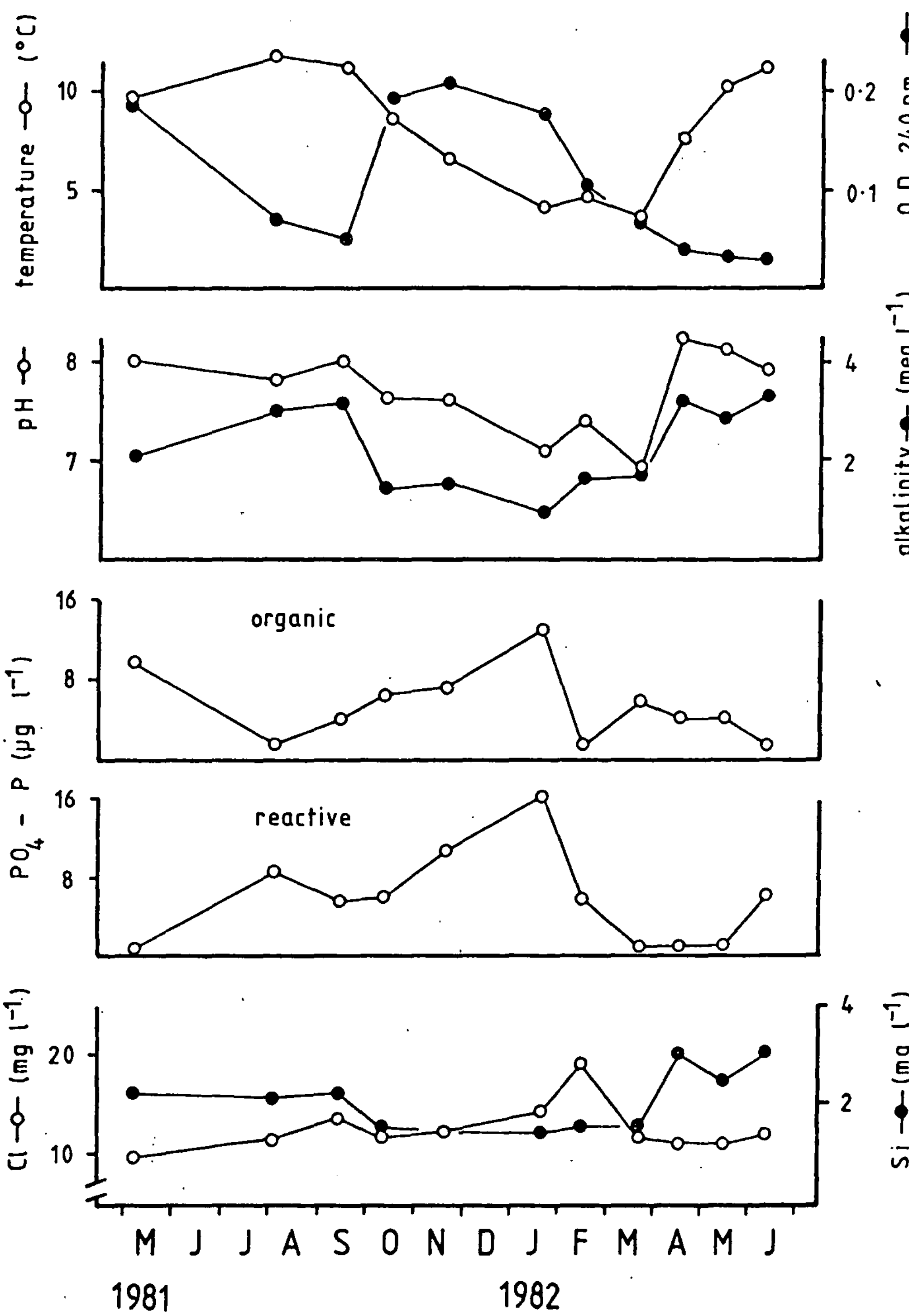

temperature (°C)
10
5
0·2
0·1
O.D. 240 nm
pH
8
7
4
2
alkalinity (meq l⁻¹)
16
organic
8
PO4 - P (µg l⁻¹)
16
reactive
8
Cl (mg l⁻¹)
20
10
4
2
Si (mg l⁻¹)
M J J A S O N D J F M A M J
1981
1982

Figure 5.03. Seasonal changes in aqueous Na, Mg, Ca, Mn and Fe (mg l^{-1}) in "High Crag Burn."

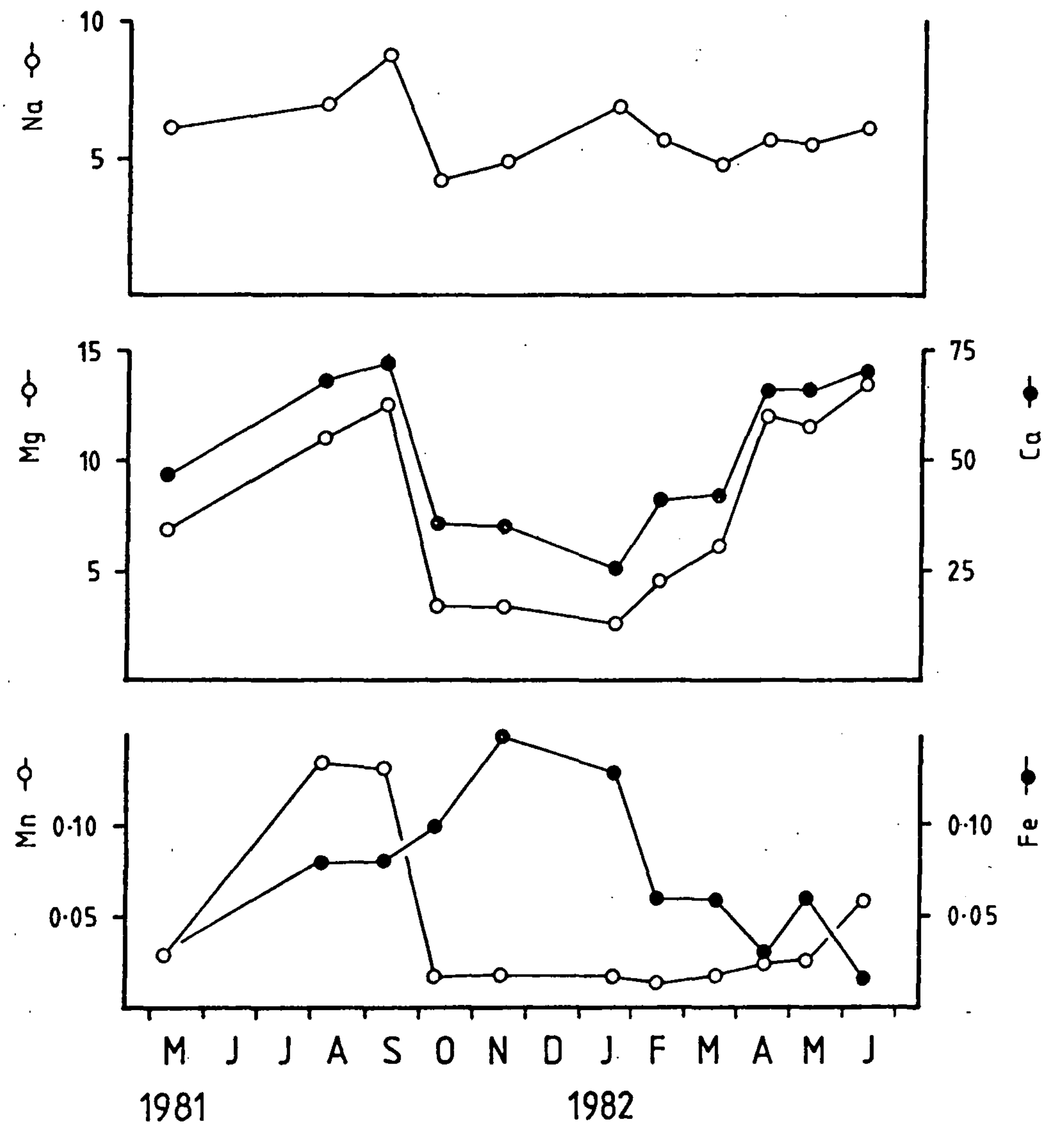
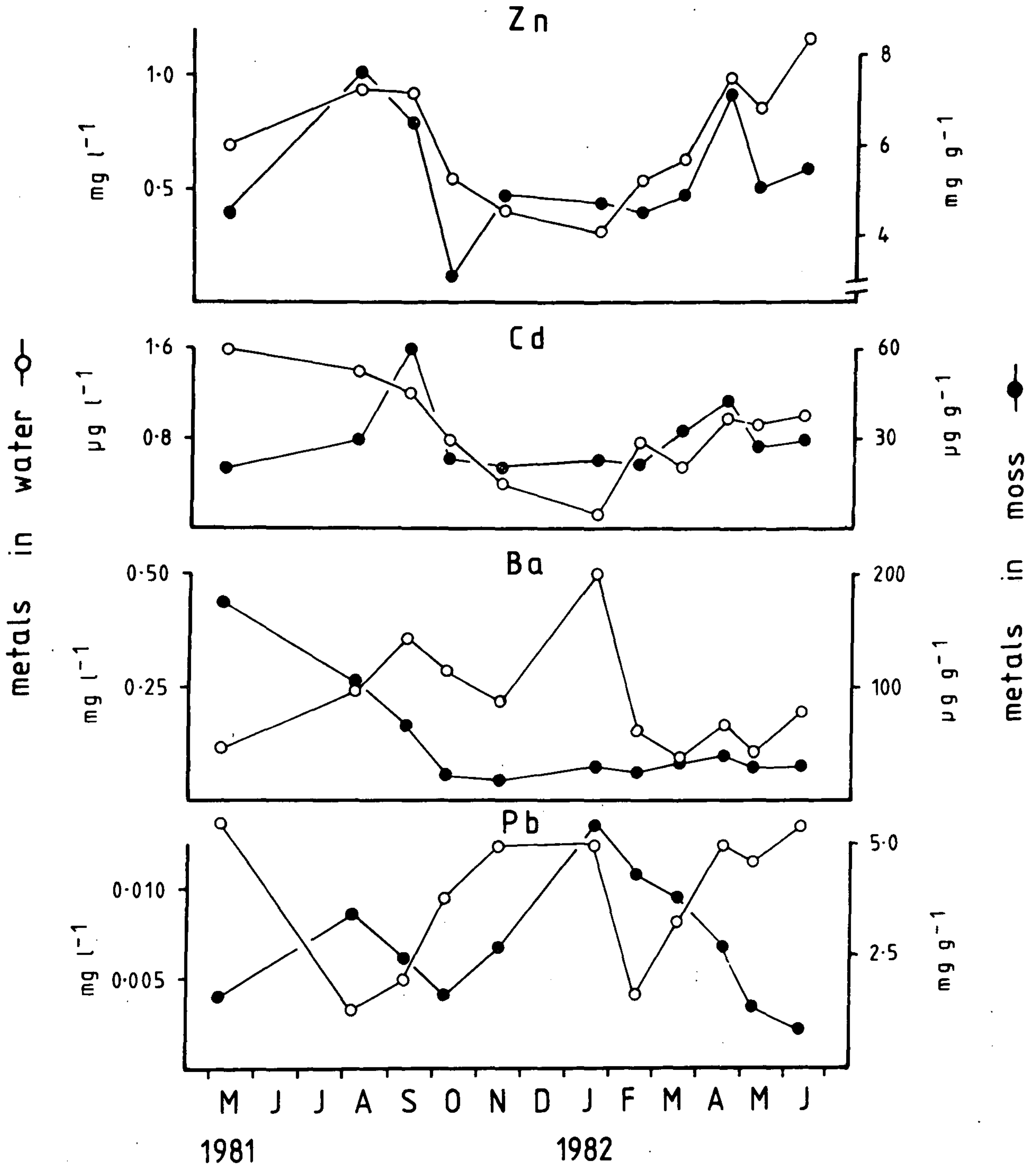

Na
10
5
Mg
15
10
5
Ca
75
50
25
Mn
0·10
0·05
Fe
0·10
0·05
M J J A S O N D J F M A M J
1981
1982

Figure 5.04. Seasonal changes in Zn, Cd, Ba and Pb in stream water and mosses in "High Crag Burn."


Zn
1·0
0·5
mg l⁻¹
8
6
4
mg g⁻¹
Cd
1·6
0·8
μg l⁻¹
60
30
μg g⁻¹
Ba
0·50
0·25
mg l⁻¹
200
100
μg g⁻¹
Pb
0·010
0·005
mg l⁻¹
5·0
2·5
mg g⁻¹
metals in water
metals in moss
M J J A S O N D J F M A M J
1981
1982

correlations for these four metals (Table 5.01) show that only zinc

Table 5.01. Temporal correlations between metals accumulated by Rhynchostegium and aqueous metals over one year in "High Crag Burn," reach 0101-05 ($n = 11$; * = $p < 0.05$).

	T	F
Zn	0.72 *	0.65 *
Cd	0.30	0.24
Ba	0.57	-0.14
Pb	-0.19	-0.43

accumulation was significantly correlated with aqueous concentrations. Seasonal variations in lead in these two components were apparently staggered. Aqueous concentrations increased in August-September, but elevated concentrations in the moss were observed in October-November. The possible causes for these differences are considered in section 5.5.

Aside from temporal factors, physical and chemical factors were considered by correlating the monthly slope (= enrichment ratio) of metal accumulation with selected variables (Table 5.02). These results indicate that the ratio for zinc in Rhynchostegium over the year correlated significantly (+) with iron in the moss. The relationship for cadmium in the moss correlated with organic phosphorus (but not reactive) and also iron in the moss. Neither of these factors were temporally correlated for barium accumulation, but a significant negative correlation appears for potassium in the moss. Manganese in the moss was significantly correlated (+) with the monthly enrichment

Table 5.02. Temporal correlations between environmental variables and enrichment ratios for Zn, Cd, Ba and Pb in Rhynchostegium over one year at "High Crag Burn," reach 0101-05 (n = 11; * = $p < 0.05$, ** = $p < 0.01$).

	Zn	Cd	Ba	Pb
temperature	-0.59	-0.53	0.05	-0.20
O.D. 240 nm	0.44	0.25	0.22	-0.12
pH	-0.53	-0.56	0.14	-0.35
total alk	-0.57	-0.51	-0.01	-0.12
filt reac PO4	0.27	0.39	-0.43	-0.02
filt org PO4	0.60 *	0.65 *	0.13	-0.27
Si	-0.55	-0.40	0.13	-0.36
Cl	0.33	0.24	-0.31	0.66 *
Na wat	0.16	0.22	-0.13	0.30
Mg wat	-0.56	-0.42	-0.02	-0.14
Ca wat	-0.59	-0.52	-0.02	-0.03
Mn wat	-0.17	-0.21	-0.16	0.38
Fe wat	0.71 *	0.50	-0.53	0.18
Mg moss	-0.36	-0.23	-0.59	0.07
K moss	0.17	0.06	-0.79 **	0.34
Ca moss	-0.31	-0.12	-0.46	0.01
Mn moss	-0.01	-0.23	-0.08	0.76 **
Fe moss	0.82 **	0.64 *	-0.26	0.56

ratio for lead, as was chloride in stream water.

5.22 Lee Springs (0289-98)

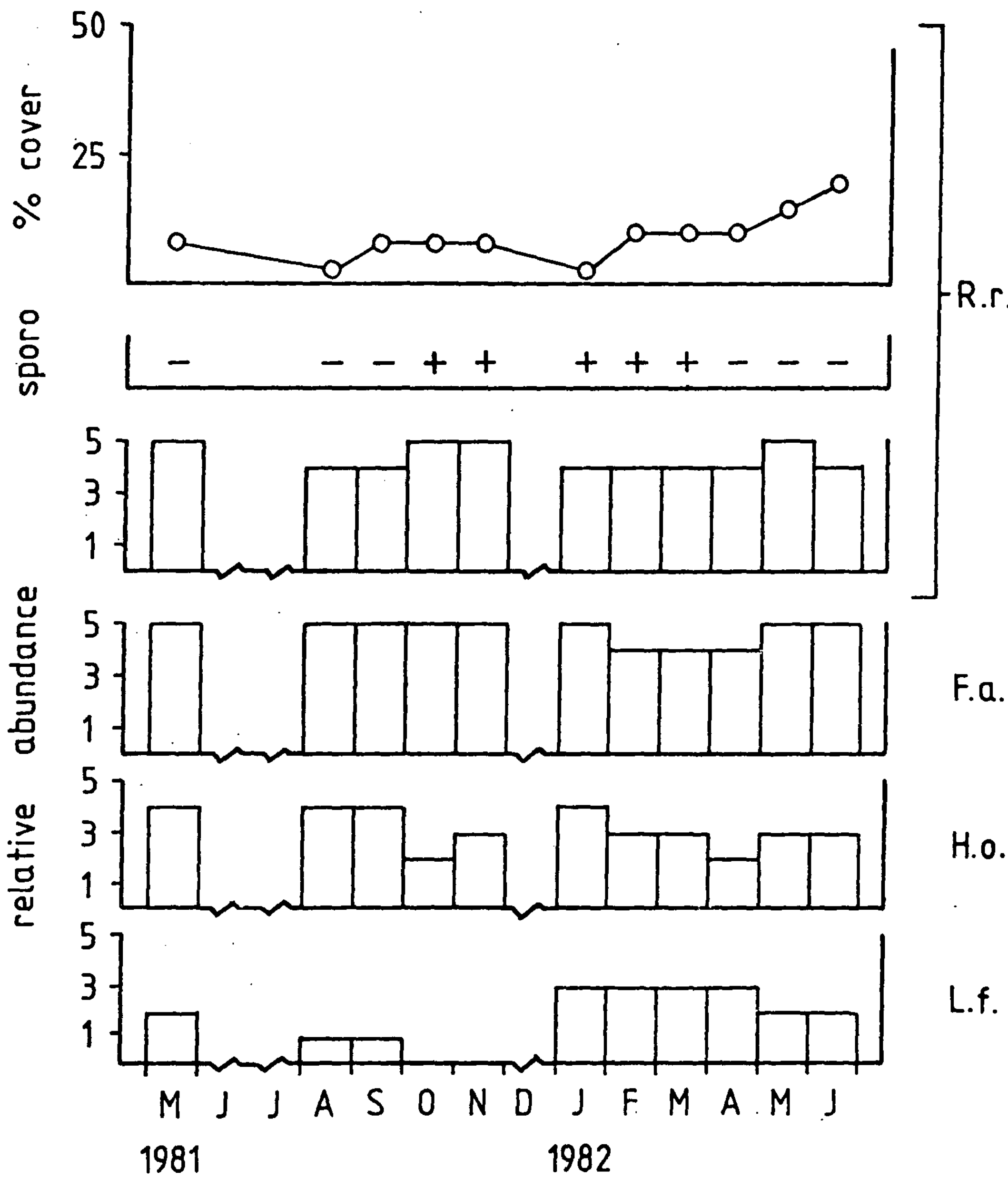
This reach has a particularly rich macrophyte flora, which includes three aquatic mosses and a macrophytic alga, Lemanea (Fig. 5.05). The dominant species in this reach were Fontinalis antipyretica and Rhynchostegium. Lemanea was least abundant in late autumn-winter (primarily chantransia stages). The cover of Rhynchostegium changed slightly over the year, with a maximum of 20% in June 1982. Sporophyte production was again limited to the autumn and winter months, and occurred on both submerged and emergent plants.

This stream, nominally recognized as a spring, was found to have a marked seasonal variation in physicochemical variables (Fig. 5.06). Temperature ranged from a minimum of 3.5 ^{o}C in March to a maximum of 11.5 ^{o}C in August. Optical density measurements revealed that water colouration increased during October and November (also increased flow). On these months the "spring" appeared distinctly peat stained. Seasonal fluctuation in pH ranged from 6.6 to 7.6. Total alkalinity varied from 0.78 - 2.82 meq l^{-1}. Silica also varied markedly over the year, but chloride was generally stable except during winter, when concentrations increased nearly fourfold.

Obvious seasonal trends in magnesium and calcium were also found (Fig. 5.07). These elements, as well as sodium to some degree, decreased during the autumn and winter months. Manganese and iron in stream water were seasonally erratic, but in general greatest concentrations were measured in autumn and spring, during higher flows.

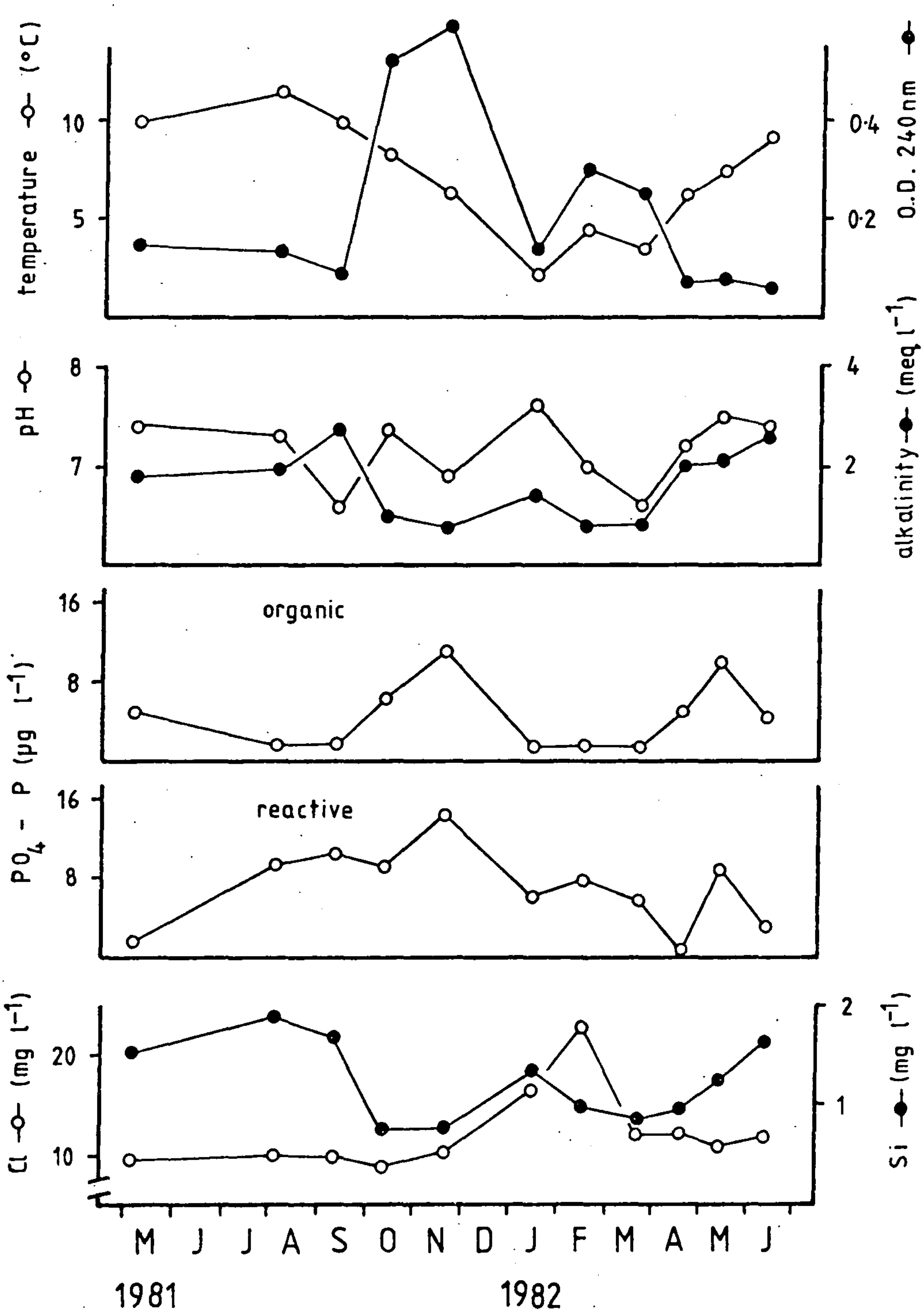
Concentrations of zinc, cadmium, barium and lead in stream water were seasonally less varied (also in lower concentrations) than in High

Figure 5.05. Seasonal changes in the relative abundance (1 - 5) of aquatic bryophytes and macroalgae, and the percent cover and sporophyte production of *Rhynchostegium* in Lee Springs (R.r. = *Rhynchostegium riparioides*, F.a. = *Fontinalis antipyretica*, L.f. = *Lemanea fluviatilis*).



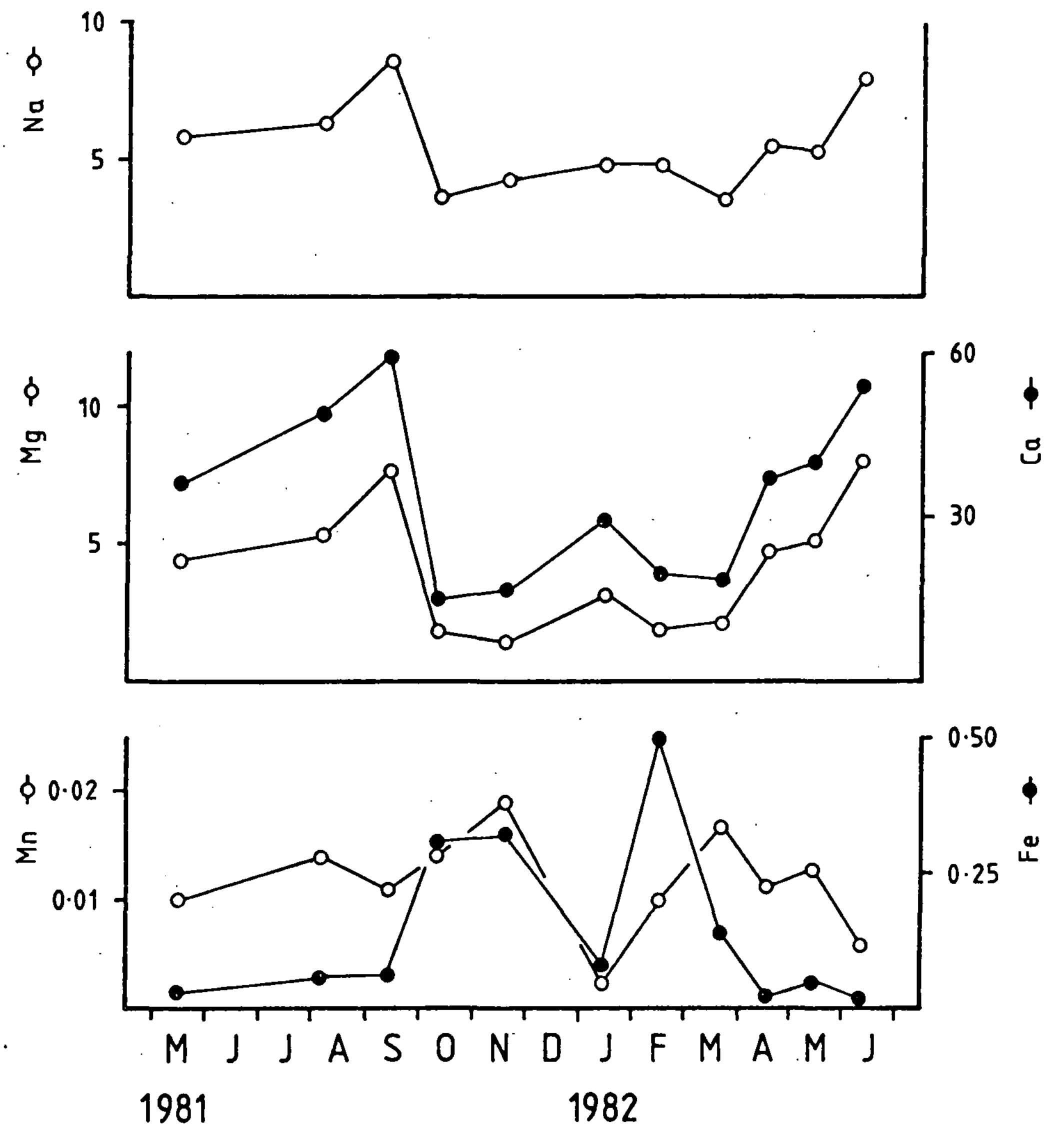
50
25
% cover
sporo
- - - + + + + + - - -
5
3
1
relative abundance
R.r.
F.a.
H.o.
L.f.
M J J A S O N D J F M A M J
1981
1982

Figure 5.06. Seasonal changes in temperature, optical density, pH, total alkalinty, phosphate (filtrable organic & reactive), chloride and silica in Lee Springs.



temperature -o- (°C)
10
5
0·4
0·2
O.D. 240nm -●-
pH -o-
8
7
4
2
alkalinity -●- (meq l^{-1})
16
8
organic
reactive
PO_4 - P (µg l^{-1})
Cl -o- (mg l^{-1})
20
10
2
1
Si -●- (mg l^{-1})
M J J A S O N D J F M A M J
1981
1982

Figure 5.07. Seasonal changes in aqueous Na, Mg, Ca, Mn and Fe (mg l^{-1}) in Lee Springs.


10
Na
5
Mg
10
5
60
Ca
30
Mn 0·02
0·01
0·50
Fe
0·25
M J J A S O N D J F M A M J
1981
1982

Crag Burn (Fig. 5.08). A fairly close correspondence was found between aqueous and accumulated metals in Rhynchostegium. The maximum filtrable zinc, 0.085 mg l^{-1}, in June 1982 coincided with a maximum of 1630 $\mu g\ g^{-1}$ in Rhynchostegium. The minimum aqueous zinc (January and March; F = 0.053 mg l^{-1}) was only about a 40% reduction from the greatest concentration measured. Abrupt peaks in aqueous barium and lead were measured on different months, although in Rhynchostegium the peaks were less extreme. Seasonal correlations (Table 5.03) indicate that zinc was

Table 5.03. Temporal correlations between metals accumulated by Rhynchostegium and aqueous metals over one year in Lee Springs reach 0289-98 ($n = 11$; * = $p < 0.05$).

	T	F
Zn	0.62*	0.64*
Cd	-0.10	0.08
Ba	0.60*	0.44
Pb	0.66	0.33

in fact the metal most strongly correlated in these two components (water and moss) on a monthly basis.

Fluctuations in aqueous calcium over the year were negatively correlated ($p < 0.05$) with the relative accumulation of zinc and barium (Table 5.04). Cadmium accumulation correlated negatively with changes in filtrable reactive phosphorus in this "spring," but none of the variables measured corresponded with the ratio for lead accumulation.

Figure 5.08. Seasonal changes in Zn, Cd, Ba and Pb in stream water and mosses in Lee Springs.

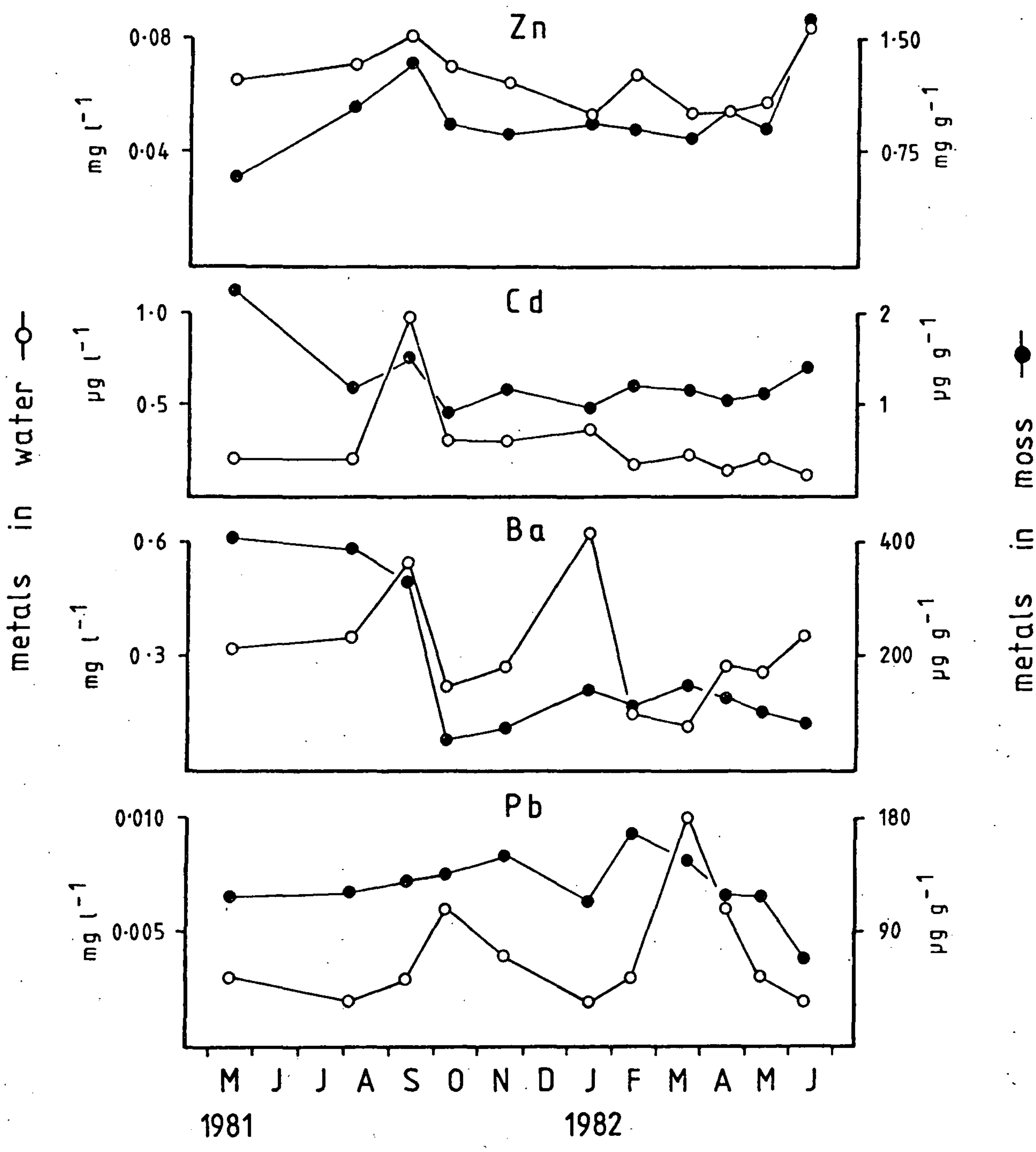

Zn
0·08
0·04
mg l⁻¹
1·50
0·75
mg g⁻¹
Cd
1·0
0·5
μg l⁻¹
2
1
μg g⁻¹
Ba
0·6
0·3
mg l⁻¹
400
200
μg g⁻¹
Pb
0·010
0·005
mg l⁻¹
180
90
μg g⁻¹
M J J A S O N D J F M A M J
1981
1982
metals in water
metals in moss

Table 5.04. Temporal correlations between environmental variables and enrichment ratios for Zn, Cd, Ba and Pb in Rhynchostegium over one year at Lee Springs reach 0289-98 (n = 11; * = $p < 0.05$).

	Zn	Cd	Ba	Pb
temperature	-0.30	0.28	0.12	0.13
O.D. 240 nm	-0.44	-0.37	-0.18	-0.19
pH	0.03	0.33	-0.38	0.27
total alk	0.36	0.25	-0.18	0.14
filt reac PO4	-0.21	-0.67 *	-0.30	0.27
filt org PO4	-0.25	0.11	-0.43	-0.25
Si	0.08	0.34	0.10	0.50
Cl	0.10	-0.03	-0.04	0.42
Na wat	0.28	0.25	-0.09	0.32
Mg wat	0.40	0.32	-0.12	0.13
Ca wat	0.34	0.25	-0.04	0.28
Mn wat	-0.37	-0.26	0.25	-0.40
Fe wat	-0.38	-0.28	-0.08	0.11
Mg moss	0.57	-0.04	-0.56	0.10
K moss	0.02	-0.22	0.11	0.25
Ca moss	0.69 *	-0.22	0.63 *	-0.35
Mn moss	-0.07	-0.10	0.34	-0.07
Fe moss	-0.29	-0.24	0.17	0.14

5.23 River West Allen (0085-50)

Aquatic bryophytes and other macrophytes were relatively sparse at this reach on the West Allen, as evidenced by the low percent cover by Rhynchostegium (Fig. 5.09). Amongst the four macrophytes present, Rhynchostegium was the most abundant. Cinclidotus mucronatus, which occurred on the upper positions of large boulders, was not submerged during periods of low flow; as such the species perhaps should not have been recorded as a "true" aquatic (sensu Holmes & Whitton, 1977a). In contrast to Lee Springs, Hygrohypnum ochraceum was most abundant in the winter.

The West Allen, a larger stream than the other three upland sites, exhibited the widest seasonal variation in physicochemical variables (Fig. 5.10). Temperature ranged from 15.8 ^{o}C in August to 1.8 ^{o}C in January. Optical density measurements had similar minima to the previous two sites, but the late autumn maximum (O.D. 240 nm = 0.720) was greater than observed elsewhere over the year. The variables pH, total alkalinity, chloride and silica also followed a seasonal pattern of spring-summer maxima and autumn-winter minima. The pH minimum of 6.4 in March 1982 was less than the minimum observed throughout the intensive survey of Rhynchostegium sites (section 4.2). Seasonal trends for both forms of phosphorus measured, in contrast, had maxima in autumn-winter.

Concentrations of several aqueous metals show distinct seasonal patterns (Fig. 5.11). Sodium, magnesium and calcium as a group were in lowest concentrations in late autumn through early spring, which were periods of low flow. Manganese and iron concentrations in stream water were greatest during this period. Peaks in concentrations of aqueous calcium in this reach (> 60 mg l^{-1}) were greater than the average of

Figure 5.09. Seasonal changes in the relative abundance (1 - 5) of aquatic bryophytes and macroalgae, and the percent cover and sporophyte production of Rhynchostegium in the River West Allen (R.r. = Rhynchostegium riparioides, H.o. = Hygrohypnum ochraceum, C.m. = Cinclidotus mucronatus, L.f. = Lemanea fluviatilis).

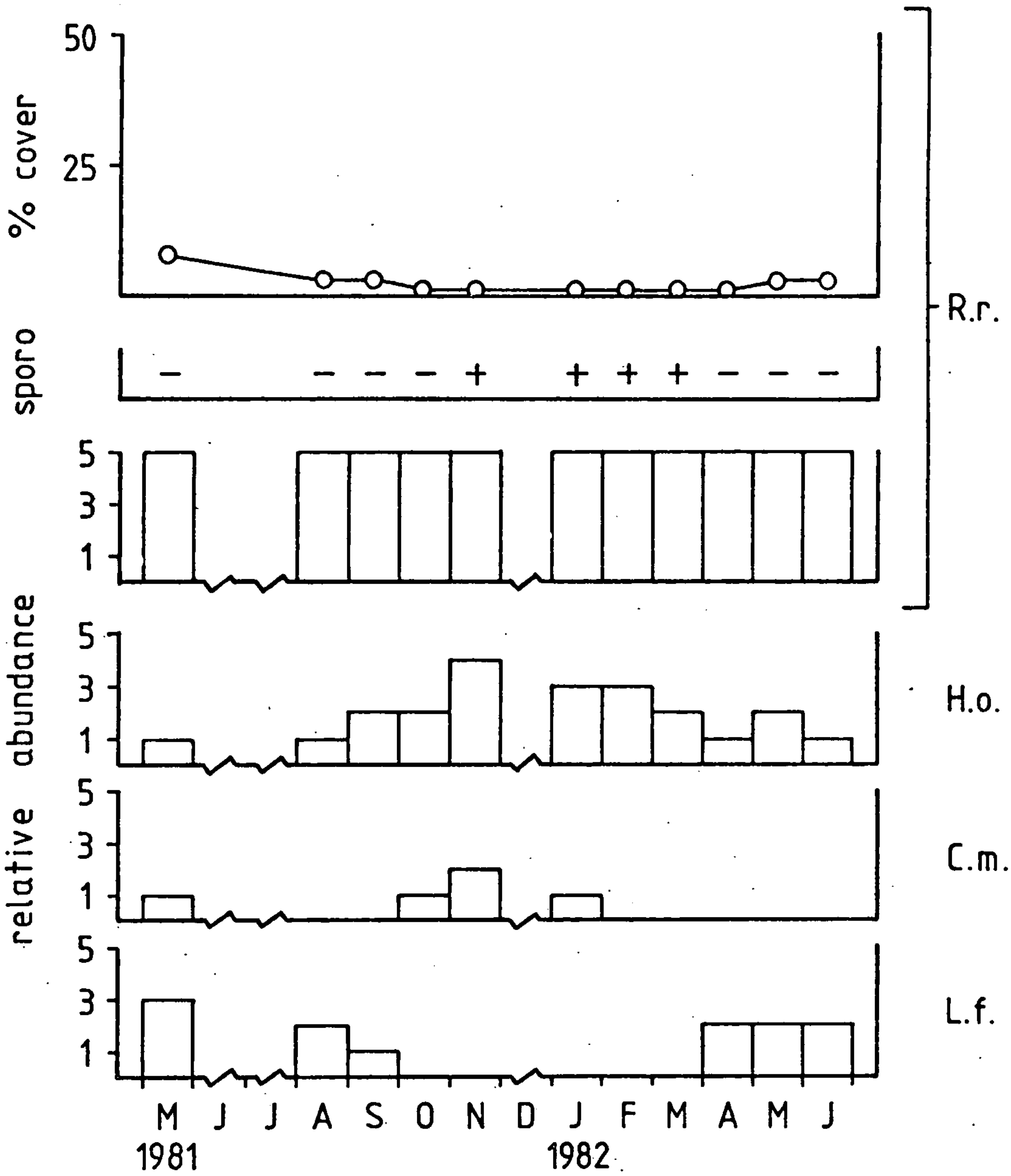
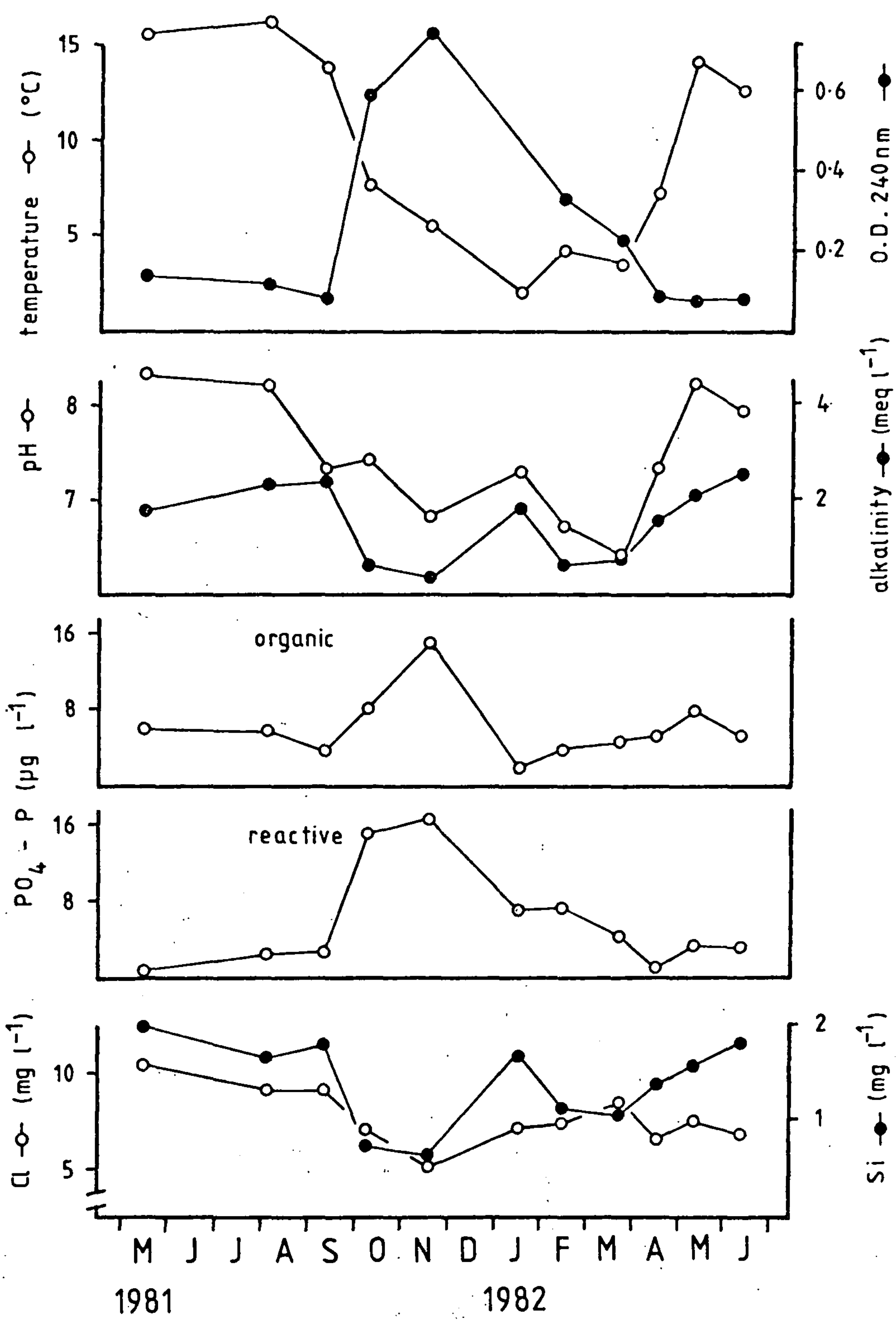
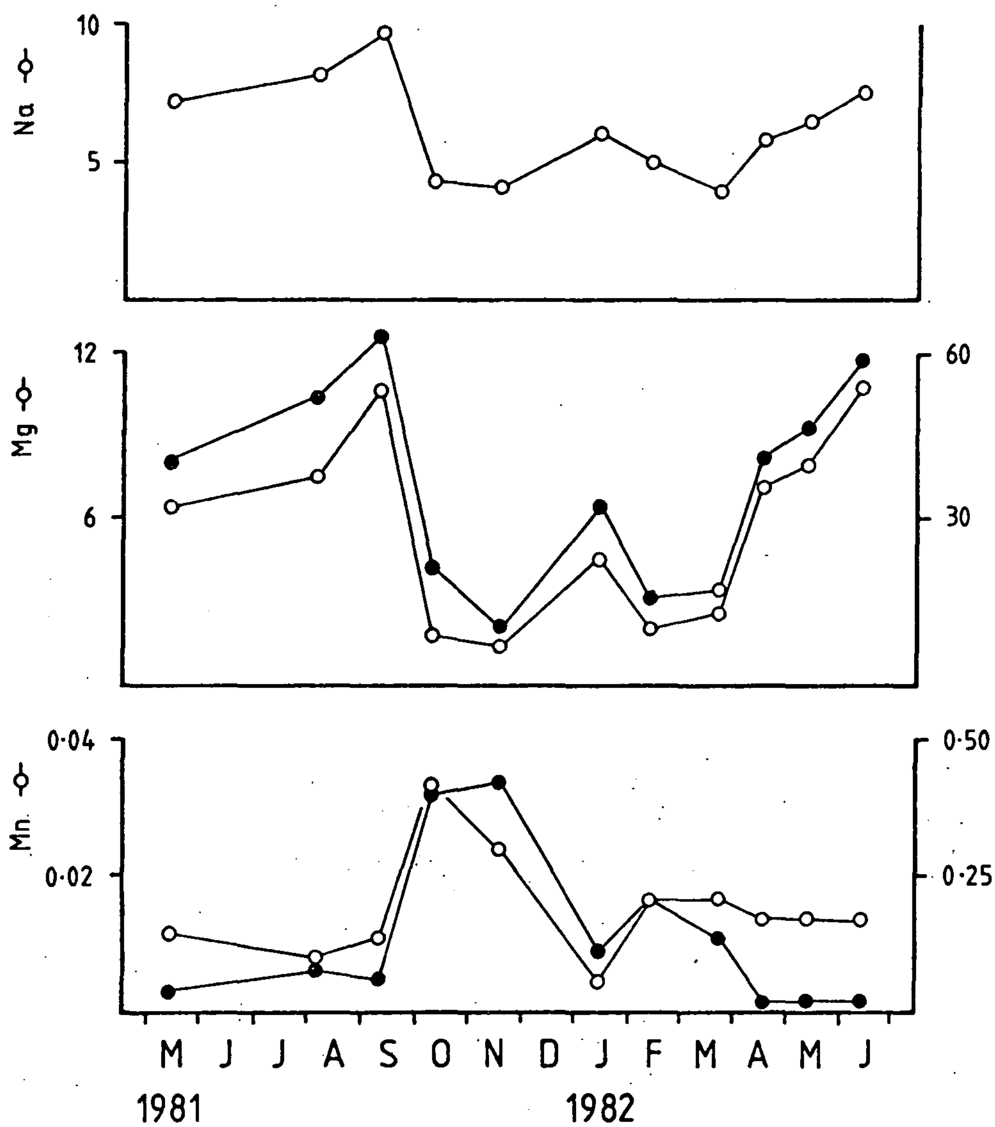

% cover
50
25
sporo
- - - - + + + + - - -
relative abundance
5
3
1
R.r.
H.o.
C.m.
L.f.
M J J A S O N D J F M A M J
1981
1982

Figure 5.10. Seasonal changes in temperature, optical density, pH, total alkalinty, phosphate (filtrable organic & reactive), chloride and silica in the River West Allen.



15
10
5
temperature -o- (°C)
0·6
0·4
0·2
O.D. 240nm -●-
8
7
pH -o-
4
2
alkalinity -●- (meq l⁻¹)
16
8
organic
16
8
reactive
PO4 - P (µg l⁻¹)
10
5
Cl -o- (mg l⁻¹)
2
1
Si -●- (mg l⁻¹)
M J J A S O N D J F M A M J
1981
1982

Figure 5.11. Seasonal changes in aqueous Na, Mg, Ca, Mn and Fe (mg l^{-1}) in the River West Allen.


10
5
Na
12
6
Mg
60
30
Ca
0·04
0·02
Mn.
0·50
0·25
Fe
M J J A S O N D J F M A M J
1981
1982

all sites from the intensive survey, while the annual minimum in the West Allen (10.4 mg l^{-1}) was similar to concentrations from softwater streams in the Lake District (section 4.22).

Concentrations of zinc, cadmium, barium and lead in the water and in Rhynchostegium also varied seasonally (Fig. 5.12). As in "High Crag Burn" (section 5.21), a close seasonal correspondence between metals in moss and water was found for zinc and cadmium. Barium and lead in these two components were less closely related. Correlations indicate (Table 5.05) that although cadmium accumulation was positively related, the

Table 5.05. Temporal correlations between metals accumulated by Rhynchostegium and aqueous metals over one year in the River West Allen, reach 0085-50 (n = 11; * = $p < 0.05$).

	T	F
Zn	0.61*	0.56
Cd	0.33	0.32
Ba	-0.48	-0.39
Pb	-0.36	-0.29

only significant correlation was with total zinc ($p < 0.05$). An increase in accumulated lead staggered two months after the pulse was measured in water, as was found for "High Crag Burn." Although similar concentrations of aqueous lead were measured in these two sites, concentrations of this metal were roughly ten times greater in Rhynchostegium populations from "High Crag Burn" than those in the West Allen.

Monthly enrichment ratios in Rhynchostegium (Table 5.06) for barium

Figure 5.12. Seasonal changes in Zn, Cd, Ba and Pb in stream water and mosses in the River West Allen.

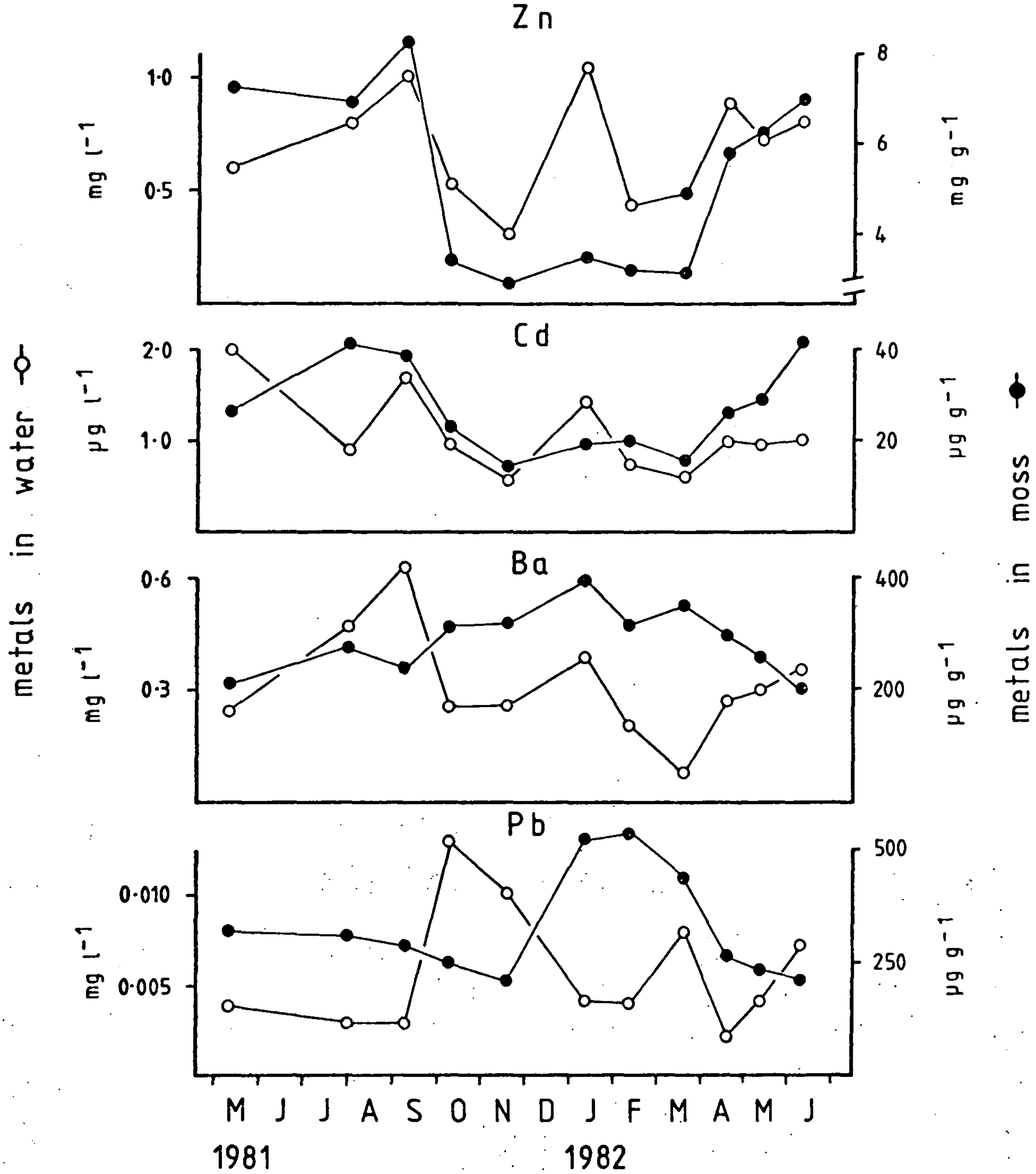

Zn
1·0
0·5
mg l⁻¹
8
6
4
mg g⁻¹
Cd
2·0
1·0
μg l⁻¹
40
20
μg g⁻¹
Ba
0·6
0·3
mg l⁻¹
400
200
μg g⁻¹
Pb
0·010
0·005
mg l⁻¹
500
250
μg g⁻¹
metals in water
metals in moss
M J J A S O N D J F M A M J
1981
1982

Table 5.06. Temporal correlations between environmental variables and enrichment ratios for Zn, Cd, Ba and Pb in Rhynchostegium over one year in the River West Allen, reach 0085-50 (n = 11; * = p < 0.05, ** = p < 0.01).

	Zn	Cd	Ba	Pb
temperature	0.75 **	0.38	-0.56	-0.13
O.D. 240 nm	-0.08	-0.15	0.20	-0.53
pH	0.51	0.23	-0.66 *	-0.03
total alk	0.22	0.32	-0.60 *	0.26
filt reac PO4	-0.29	-0.20	0.08	-0.51
filt org PO4	0.36	0.18	-0.01	-0.71 *
Si	0.32	0.03	-0.45	0.41
Cl	0.46	-0.15	0.03	0.25
Na wat	0.34	0.26	-0.64 *	0.30
Mg wat	0.33	0.37	-0.56	0.15
Ca wat	0.29	0.36	-0.62 *	0.18
Mn wat	0.04	-0.07	0.26	-0.65 *
Fe wat	-0.23	-0.17	0.20	-0.47
Mg moss	0.07	0.32	-0.54	-0.36
K moss	-0.19	0.24	-0.55	-0.27
Ca moss	-0.38	0.42	-0.37	-0.01
Mn moss	0.47	0.61 *	-0.20	0.15
Fe moss	-0.29	-0.32	0.66 *	0.28

and lead correlated significantly with two environmental factors. With barium, aqueous sodium and calcium are negatively correlated, while seasonal fluctuations in organic phosphorus and aqueous manganese correlated negatively with lead accumulation. The analysis also suggests that increased temperature was related to the monthly accumulation of zinc. Manganese in the moss correlated with greater cadmium accumulation.

5.24 "Race Fell Burn" (0310-90)

The fourth upland stream studied, "Race Fell Burn," had a fairly diverse aquatic bryophyte flora, although Rhynchostegium was by far the most abundant species present (Fig. 5.13). Large, dense stands of this species ranged in percent cover from 20% in September during a very low flow when most of the plants were emergent, to 75% of the streambed, in May 1981 and March-April 1982. Sporophytes were produced by this population between October and April, although principally by plants out of water. Fontinalis antipyretica was the next most abundant macrophyte, although less abundant than Rhynchostegium. Chiloscyphus polyanthos was observed on nine months of the year and Scapania undulata on only four months.

Seasonal fluctuations in several physicochemical variables were less extreme than in the three other upland sites (Fig. 5.14). Silica levels ranged between about 2 - 3 mg l^{-1} and chloride between 6 - 10 mg l^{-1}. Further, pH was restricted to between 7.0 - 7.8, despite the comparatively low total alkalinity (TA) year round (< 1.0 meq l^{-1}). However, TA itself declined by about 70% during the winter. Also unlike the other upland streams, concentrations of organic phosphorus decreased over the autumn-winter period. Concentrations of filtrable

Figure 5.13. Seasonal changes in the relative abundance (1 - 5) of aquatic bryophytes and macroalgae, and the percent cover and sporophyte production of Rhynchostegium in "Race Fell Burn" (R.r. = Rhynchostegium riparioides, C.p. = Chiloscyphus polyanthos, S.u. = Scapania undulata B.p. = Bryum pallens, F.a. = Fontinalis antipyretica.

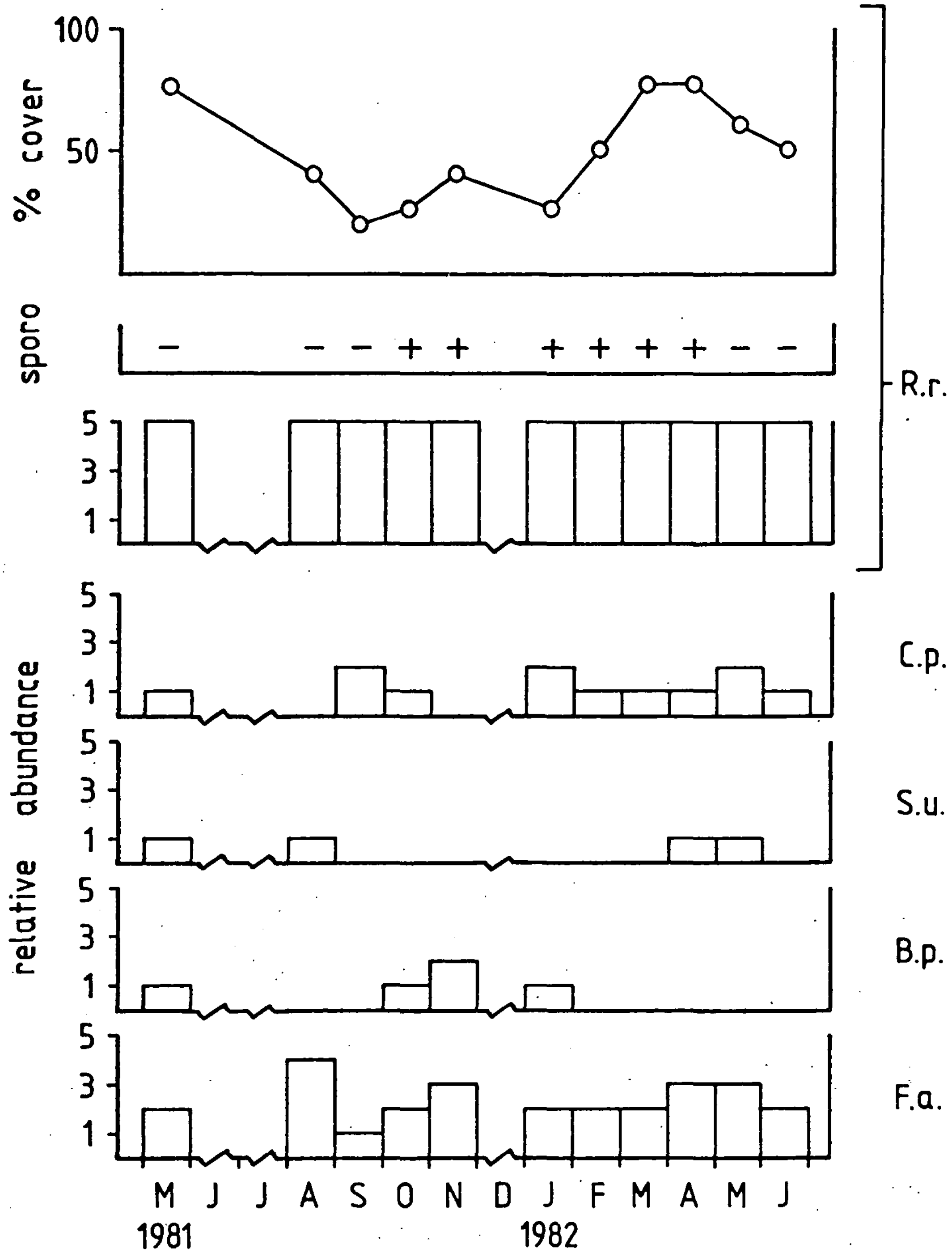
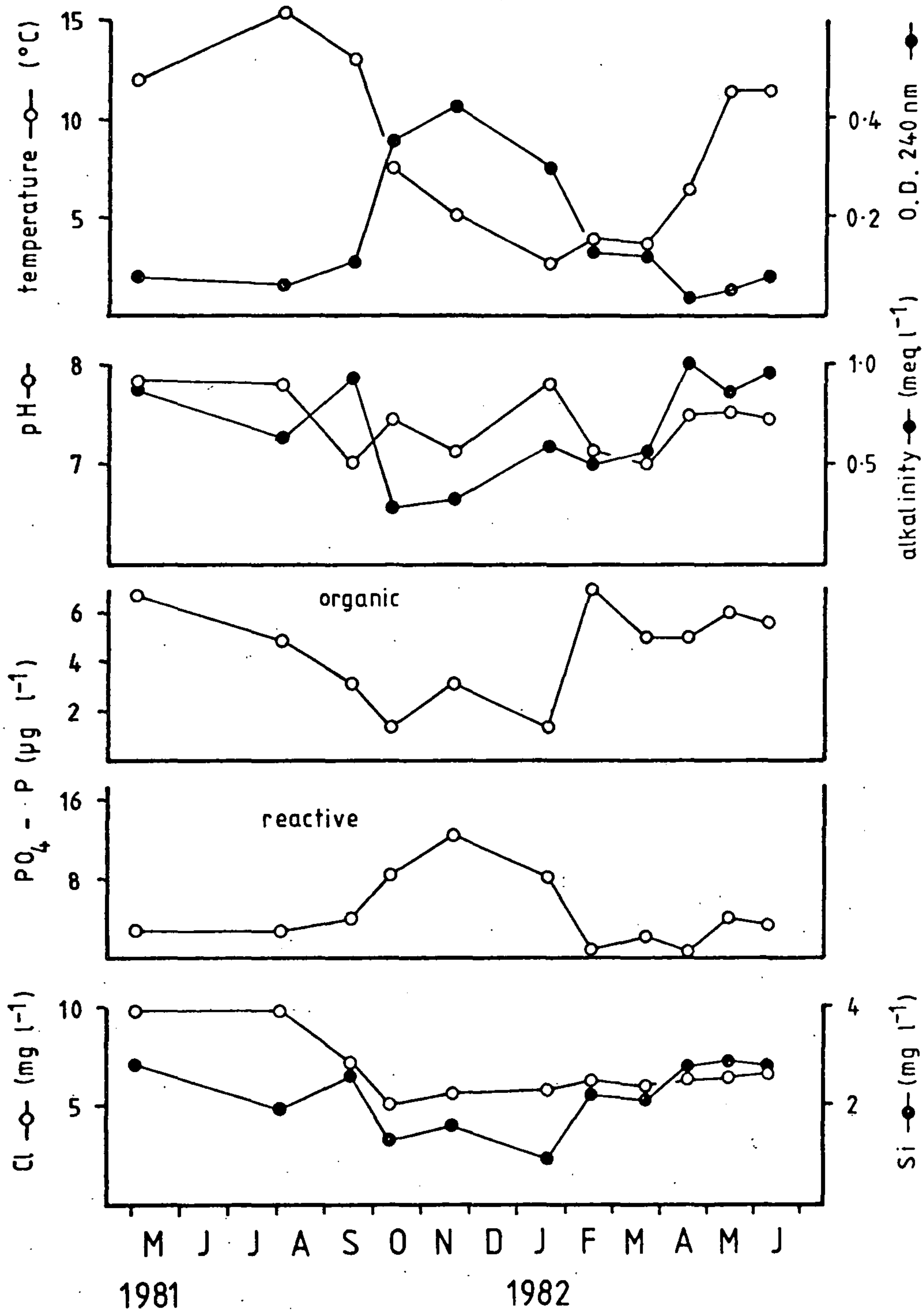

100
50
% cover
sporo
– – – + + + + + + – –
R.r.
5
3
1
C.p.
S.u.
B.p.
F.a.
relative abundance
M J J A S O N D J F M A M J
1981
1982

Figure 5.14. Seasonal changes in temperature, optical density, pH, total alkalinty, phosphate (filtrable organic & reactive), chloride and silica in "Race Fell Burn."



temperature —o— (°C)
15
10
5
O.D. 240 nm —●—
0·4
0·2
pH —o—
8
7
alkalinity —●— (meq l^{-1})
1·0
0·5
organic
6
4
2
PO$_4$ - P (µg l^{-1})
16
8
reactive
Cl —o— (mg l^{-1})
10
5
Si —●— (mg l^{-1})
4
2
M J J A S O N D J F M A M J
1981
1982

reactive phosphorus, in contrast, were greatest during this period. Magnesium and calcium concentrations followed a distinct seasonal pattern in Race Fell Burn (Fig. 5.15) and were the lowest amongst the four upland streams studied. No seasonal trend was apparent for concentrations of aqueous sodium. Manganese and iron in stream water were somewhat seasonally erratic, although greatest concentrations tended to occur during autumn and winter. Although there was little obvious peat colouration in the water, increases in aqueous iron correspond fairly closely with greater optical density (Fig. 5.14).

Zinc, cadmium, barium and lead concentrations in water and mosses were the lowest amongst the upland streams (Fig. 5.16). Although concentrations were low, an apparently close correspondence over the year between aqueous metals and metals in Rhynchostegium was observed. Temporal correlations indicate they (Table 5.07) were significant only

Table 5.07. Temporal correlations between metals accumulated by Rhynchostegium and aqueous metals over one year in "Race Fell Burn," reach 0310-90 ($n = 11$; * = $p < 0.05$, ** = $p < 0.01$).

	T	F
Zn	0.19	0.44
Cd	0.62 *	0.76 **
Ba	0.56	0.63 *
Pb	0.30	0.52

for cadmium and barium, however. The relationship between zinc in stream water and in the moss appears to be staggered (see also section 5.5). An increase in aqueous cadmium in the stream during a near drought in September resulted in an increase in this metal in the moss

Figure 5.15. Seasonal changes in aqueous Na, Mg, Ca, Mn and Fe (mg l^{-1}) in "Race Fell Burn."

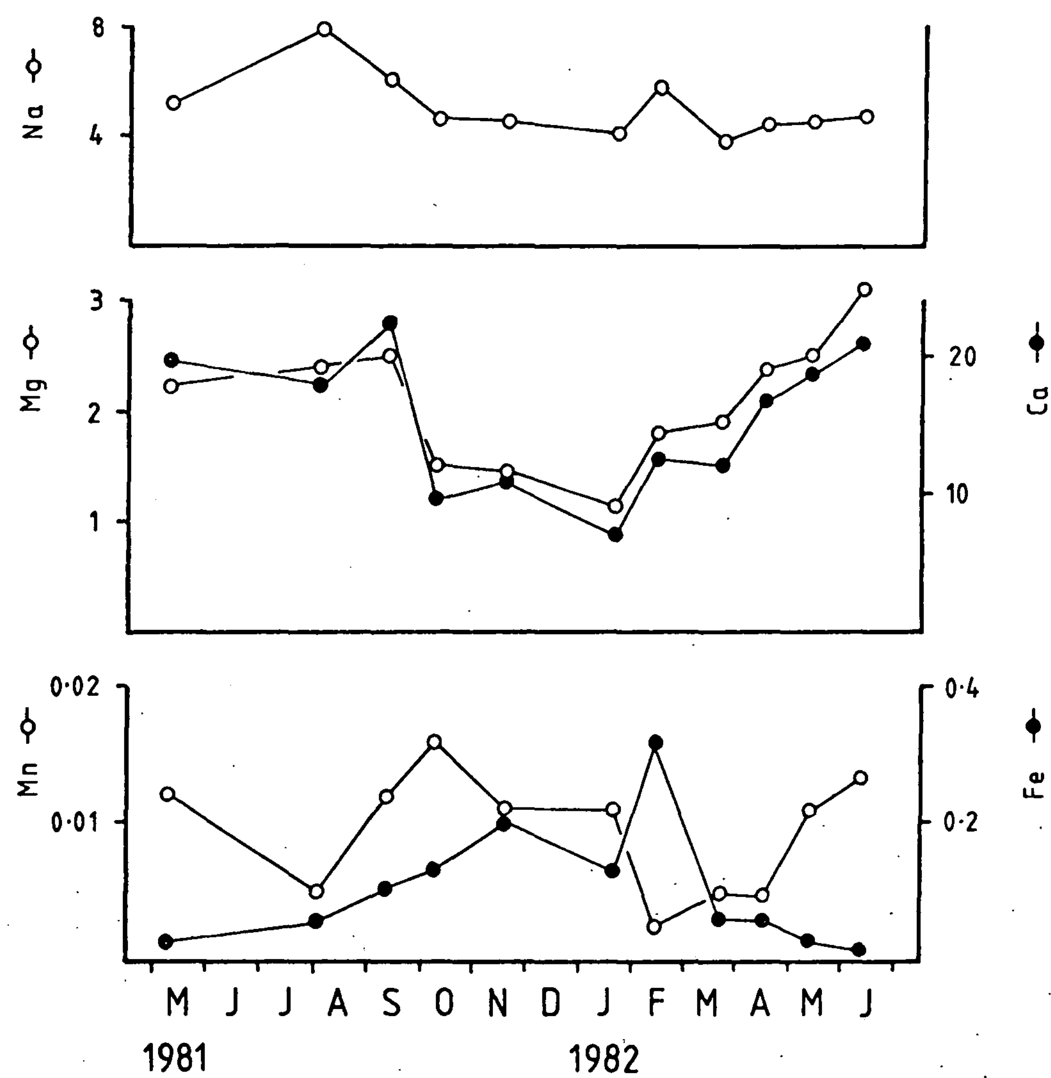
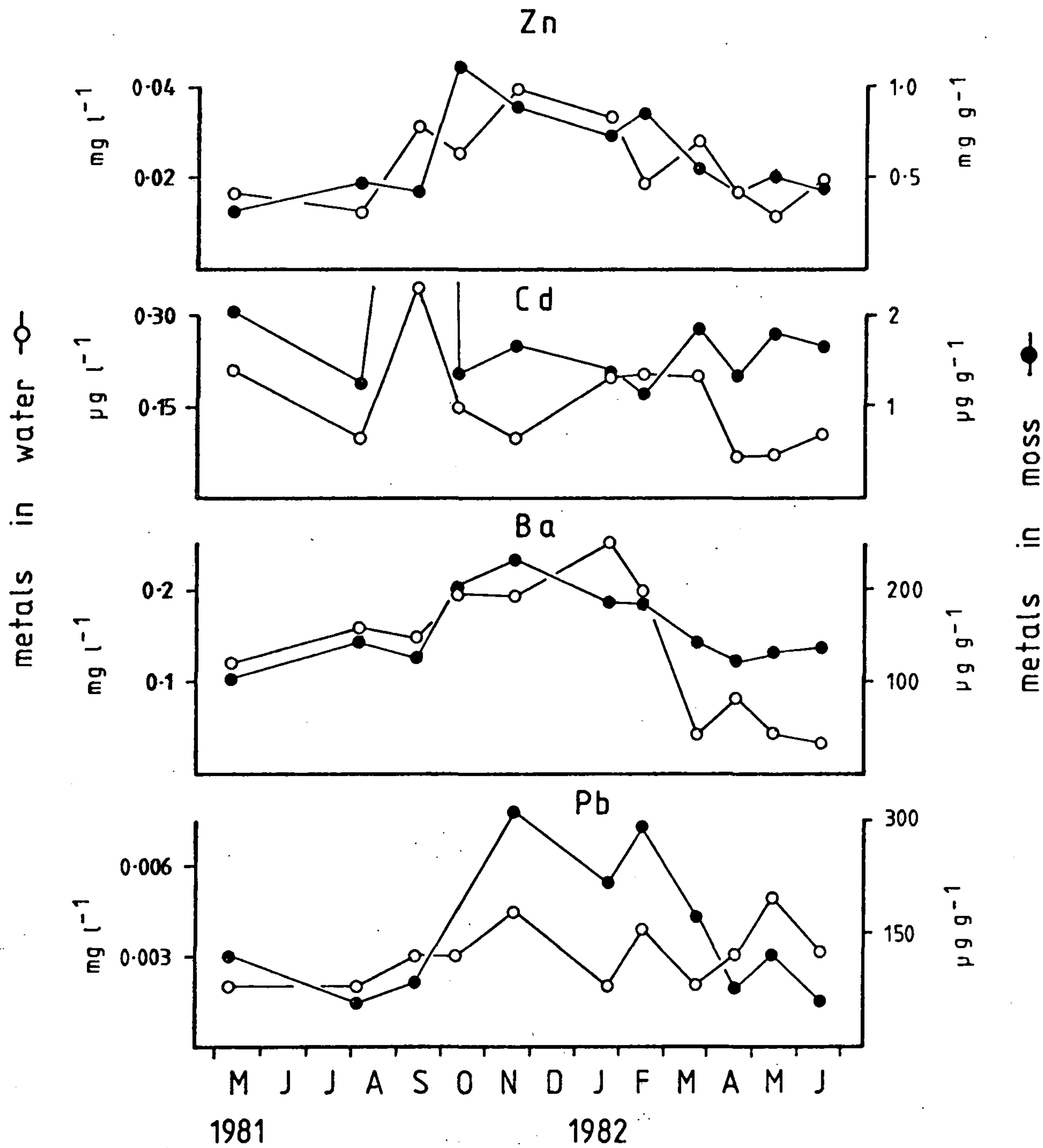

8
4
Na
3
2
1
Mg
20
10
Ca
0·02
0·01
Mn
0·4
0·2
Fe
M J J A S O N D J F M A M J
1981
1982

Figure 5.16. Seasonal changes in Zn, Cd, Ba and Pb in stream water and mosses in "Race Fell Burn."


Zn
Cd
Ba
Pb
metals in water
metals in moss
mg l⁻¹
μg l⁻¹
mg g⁻¹
μg g⁻¹
0·04
0·02
1·0
0·5
0·30
0·15
2
1
0·2
0·1
200
100
0·006
0·003
300
150
M J J A S O N D J F M A M J
1981
1982

(29.5 $\mu g\ g^{-1}$), comparable to West Allen population. Otherwise, concentrations of "heavy metals," in general, were low. In contrast to the previous three sites, barium in stream water and moss correlated significantly over the year ($p < 0.05$).

A correlation between monthly enrichment ratios and environmental factors (Table 5.08) found no significant relations existed for zinc, cadmium or barium. However, several variables were correlated with fluctuations in lead accumulation. The stongest of these ($p < 0.01$) were negative correlations with aqueous magnesium and calcium. Returning to the seasonal plot (Fig. 5.16), the higher accumulation of lead (relative to aqueous concentrations) by Rhynchostegium between November-February was a period of lower temperatures and reduced magnesium and calcium. Further, manganese and iron in the plants were also significantly correlated, positively.

5.3 Descriptive statistics for three lowland rivers

Data for three lowland rivers, all from region VI, the Durham Coalfield are given in this section. Two reaches are on the River Team, the first at Kyo Heaugh (0024-05) and the second at Causey Arch (0024-20). These sites are intended to represent conditions above and below the input of several industrial effluents and sewage works, where zinc contamination has been shown to have resulted (Wehr et al., 1981). These two locations were sampled on 13 months of the 14 month period in 1981 and 1982. The third site is a lowland reach on the River Wear (0008-65), downstream of several sewage works and nutrient-rich tributaries and just upstream of Durham city. As this site was frozen over in winter, it was sampled on 10 out of the 14 months. Data are

Table 5.08. Temporal correlations between environmental variables and enrichment ratios for Zn, Cd, Ba and Pb in Rhynchostegium over one year at "Race Fell Burn," reach 0310-90 (n = 11; * = p < 0.05, ** = p < 0.01, *** = p < 0.001).

	Zn	Cd	Ba	Pb
temperature	-0.01	0.44	0.06	-0.76 **
O.D. 240 nm	0.09	-0.20	-0.36	0.46
pH	0.14	-0.41	-0.24	0.01
total alk	-0.43	0.42	0.30	-0.56
filt reac PO4	0.01	-0.03	-0.22	0.25
filt org PO4	0.01	-0.09	0.24	-0.32
Si	-0.19	0.37	0.40	-0.66 *
Cl	-0.16	-0.07	-0.03	-0.34
Na wat	0.16	0.25	-0.38	-0.36
Mg wat	-0.17	0.38	0.54	-0.81 ***
Ca wat	-0.26	0.54	0.29	-0.77 **
Mn wat	-0.07	0.22	0.04	-0.14
Fe wat	0.35	-0.14	-0.48	0.43
Mg moss	0.03	0.46	0.15	-0.34
K moss	-0.01	0.51	-0.14	-0.08
Ca moss	-0.12	0.28	0.44	-0.57
Mn moss	0.13	-0.33	-0.30	0.62 *
Fe moss	0.43	-0.37	-0.35	0.61 *

presented in a similar format to the section on upland streams, but include metal accumulation data for _Amblystegium riparium_ in the Team (0024-20) and _Fontinalis antipyretica_ in the Wear (0008-65), in addition to _Rhynchostegium_.

5.31 River Team, Kyo Heaugh (0024-05)

Two macrophytic algal species and two aquatic mosses were regularly found in this stream (Fig. 5.17). _Rhynchostegium_ was generally sparse, never exceeding 1 - 5% cover, although the dominant macrophyte most months of the year. In a period beginning February 1982, oil was dumped into a tributary which enters the river about 200 m upstream of this reach, which continued through May. Subsequently, the relative abundance of _Rhynchostegium_ declined and the green alga _Stigeoclonium_ increased. There was a noticeable improvement in the cover and growth form of _Rhynchostegium_ in June 1982, when oil was apparently no longer being released into the river. _Amblystegium riparium_ was not found during the months of February through April and _Cladophora glomerata_ was not in evidence between December and April. At no time was _Rhynchostegium_ found to produce sporophytes.

Physicochemical variables were seasonally unpredictable (Fig. 5.18). The most obvious change was in January when a pulse in several anions (phosphates and chloride) and optical density was observed. At this time a heavy growth of sewage fungus covered much of the stream bottom. However, silica and pH did not change. There was little evidence of gradual seasonal variation, such as maxima in summer and minima in winter, or the reverse. Total alkalinity always remained > 1.0 meq l^{-1} and pH usually > 7.0. Chloride was usually > 40 mg l^{-1} and with the exception of January, varied little.

Figure 5.17. Seasonal changes in the relative abundance (1 - 5) of aquatic bryophytes and macroalgae, and the percent cover and sporophyte production of Rhynchostegium in the River Team, Kyo Heaugh (R.r. = Rhynchostegium riparioides, A.r. = Amblystegium riparium, C.g. = Cladophora glomerata, S.t. = Stigeoclonium tenue).

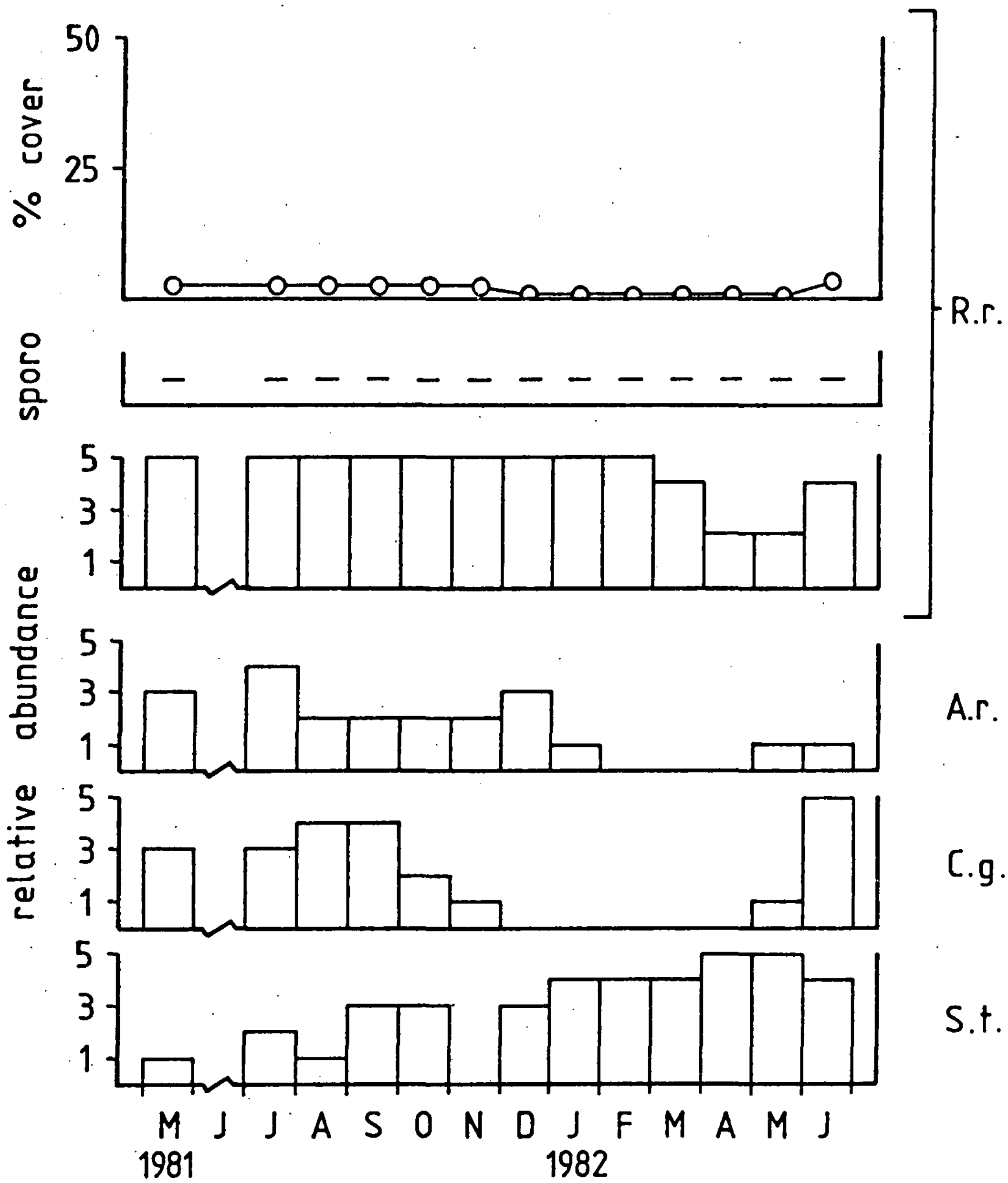
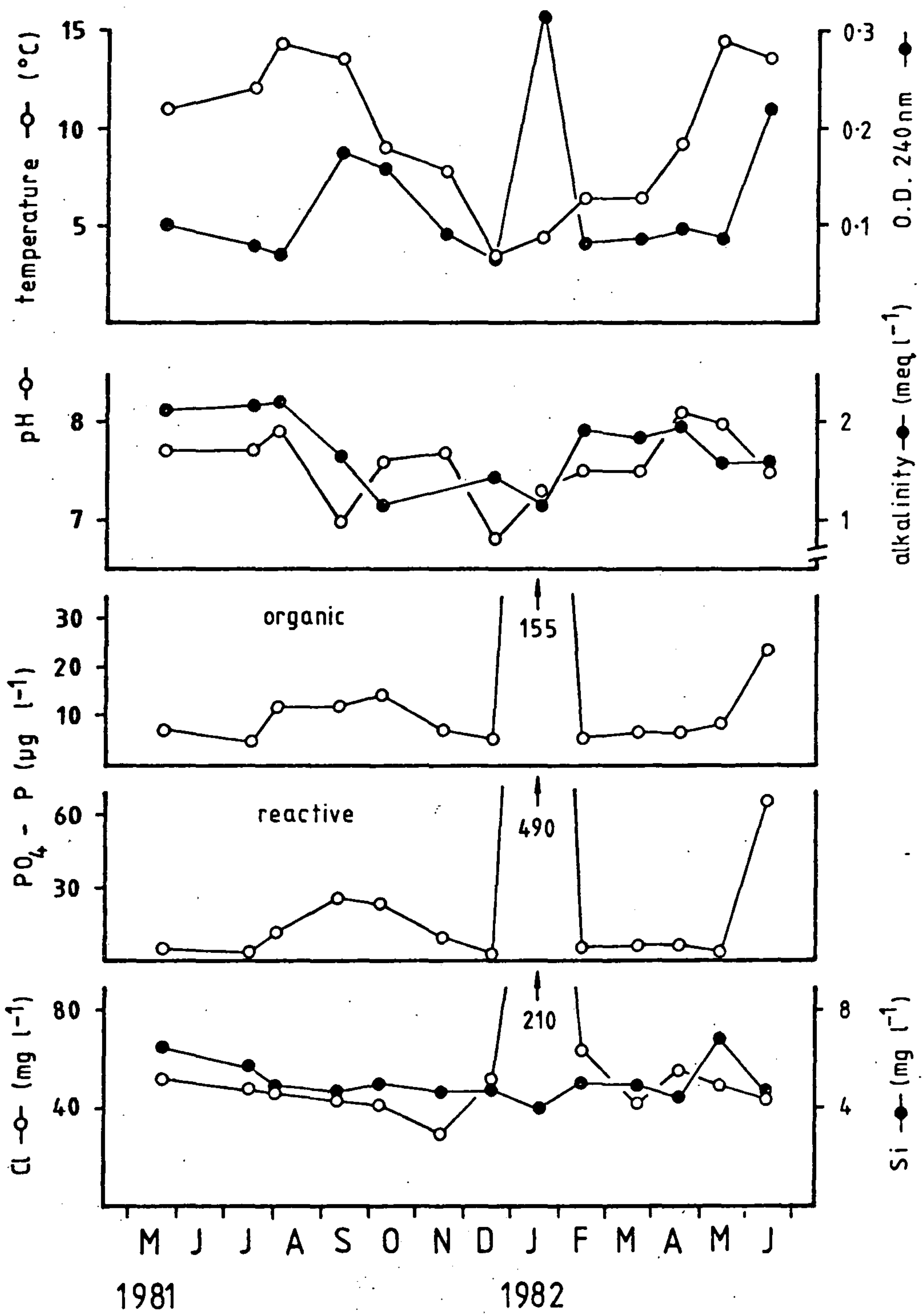

50
25
% cover
sporo
5
3
1
relative abundance
R.r.
A.r.
C.g.
S.t.
M J J A S O N D J F M A M J
1981
1982

Figure 5.18. Seasonal changes in temperature, optical density, pH, total alkalinty, phosphate (filtrable organic & reactive), chloride and silica in the River Team, Kyo Heaugh.



15
10
5
temperature -o- (°C)
0·3
0·2
0·1
O.D. 240nm -●-
pH -o-
8
7
2
1
alkalinity -●- (meq l⁻¹)
organic
155
30
20
10
PO₄ - P (µg l⁻¹)
reactive
490
60
30
210
80
40
Cl -o- (mg l⁻¹)
8
4
Si -●- (mg l⁻¹)
M J J A S O N D J F M A M J
1981
1982

Concentrations of magnesium, calcium and manganese varied according to a noticeable seasonal pattern (Fig. 5.19). Concentrations of these elements decreased, although somewhat erratically, during autumn-winter and were greatest in spring-summer months. Sodium concentrations in stream water varied little over the year, but did not peak during January along with several of the anions. Aqueous iron varied erratically without any apparent seasonal trend.

Aqueous zinc remained < 0.05 mg l^{-1} throughout most of the year (Fig. 5.20); concentrations of other metals were similarly low. Metal accumulation followed fairly closely temporal changes in aqueous concentrations, at least for zinc, cadmium and lead. Barium in Rhynchostegium, however, was found to show little relation with fluctuations in aqueous concentrations. Correlations (Table 5.09) in

Table 5.09. Temporal correlations between metals accumulated by Rhynchostegium and aqueous metals over one year in the River Team, Kyo Heaugh, reach 0024-05 (n = 13; * = p < 0.05, ** = p < 0.01).

	T	F
Zn	0.54	0.72 **
Cd	-0.25	0.15
Ba	-0.67 *	-0.64 *
Pb	-0.03	0.18

fact indicate a significant negative seasonal relation for barium. Only zinc accumulation by the moss was positively correlated with temporal changes in aqueous concentrations. In the period when oil was released into the stream (February - May), there was little evidence of any increase in concentrations of aqueous metals.

Figure 5.19. Seasonal changes in aqueous Na, Mg, Ca, Mn and Fe (mg l^{-1}) in the River Team, Kyo Heaugh.

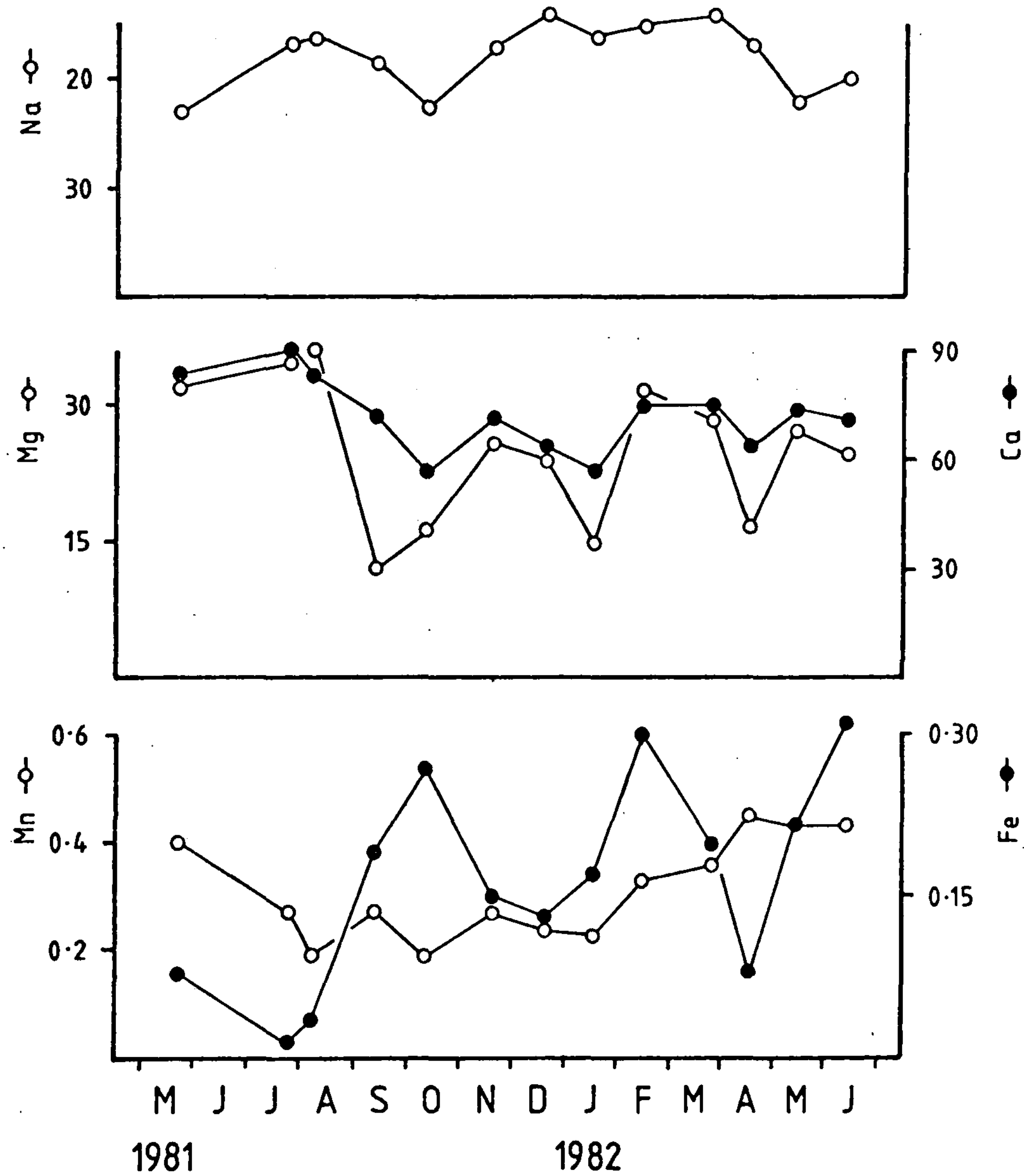
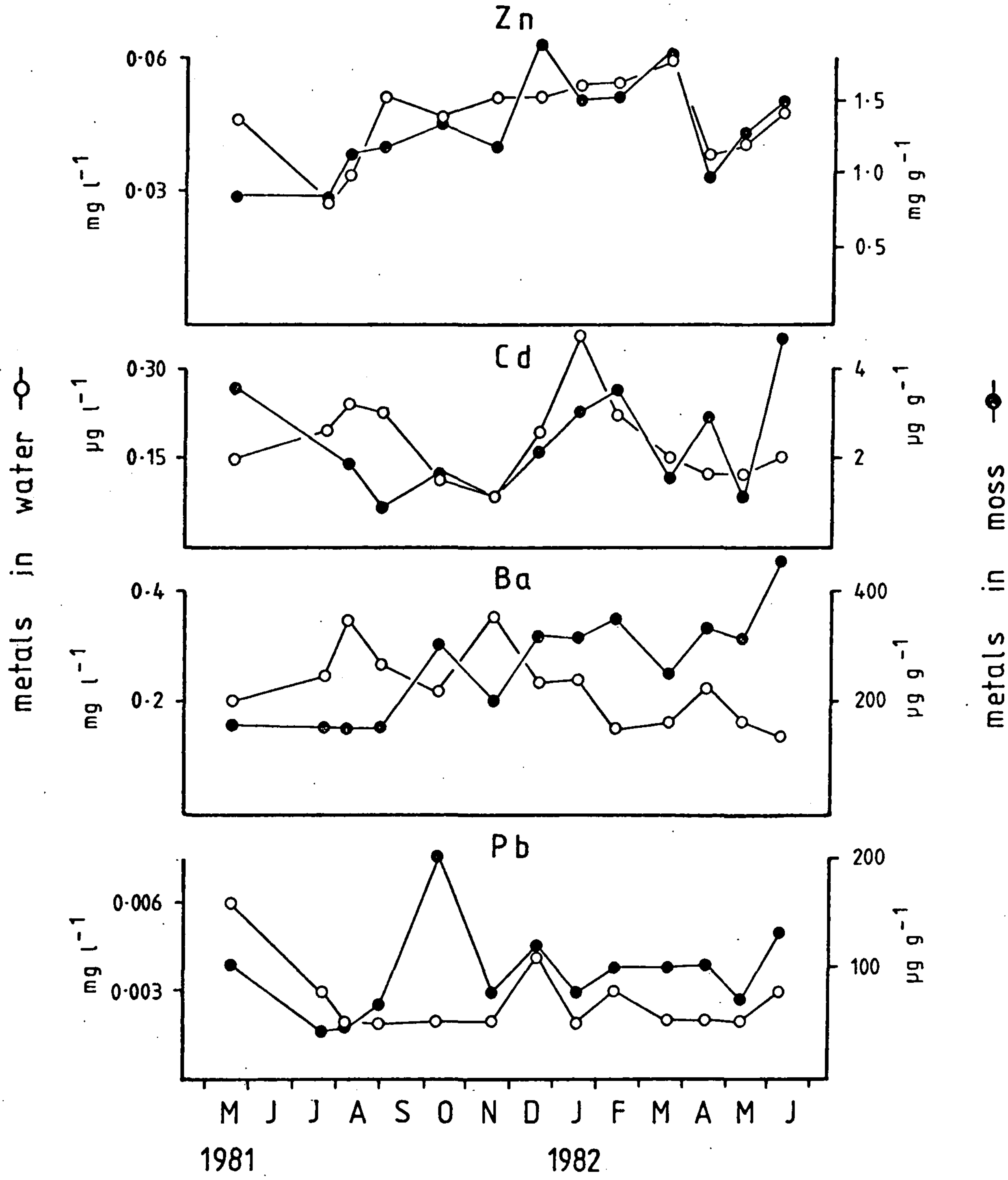

Na
20
30
Mg
30
15
90
60
30
Ca
Mn
0·6
0·4
0·2
0·30
0·15
Fe
M J J A S O N D J F M A M J
1981
1982

Figure 5.20. Seasonal changes in Zn, Cd, Ba and Pb in stream water and mosses in the River Team, Kyo Heaugh.



Zn
0·06
0·03
mg l⁻¹
1·5
1·0
0·5
mg g⁻¹
Cd
0·30
0·15
µg l⁻¹
4
2
µg g⁻¹
Ba
0·4
0·2
mg l⁻¹
400
200
µg g⁻¹
Pb
0·006
0·003
mg l⁻¹
200
100
µg g⁻¹
metals in water
metals in moss
M J J A S O N D J F M A M J
1981
1982

Although aqueous zinc was found to be a principal factor leading to increased zinc accumulation by Rhynchostegium (Table 5.09), no other environmental factors appear to be involved (Table 5.10). Several variables correlated with changes in enrichment ratios. Barium accumulation (correlated negatively with barium fluctuations in stream water), was apparently strongly influenced ($p < 0.01$) by concentrations of aqueous iron and by manganese in the moss; the stream originates from an iron-rich spring only 1 km upstream. Cadmium accumulation also correlated with greater manganese in Rhynchostegium. Lead accumulation was apparently reduced by water hardness, as evidenced by negative correlations with aqueous magnesium and calcium and total alkalinity.

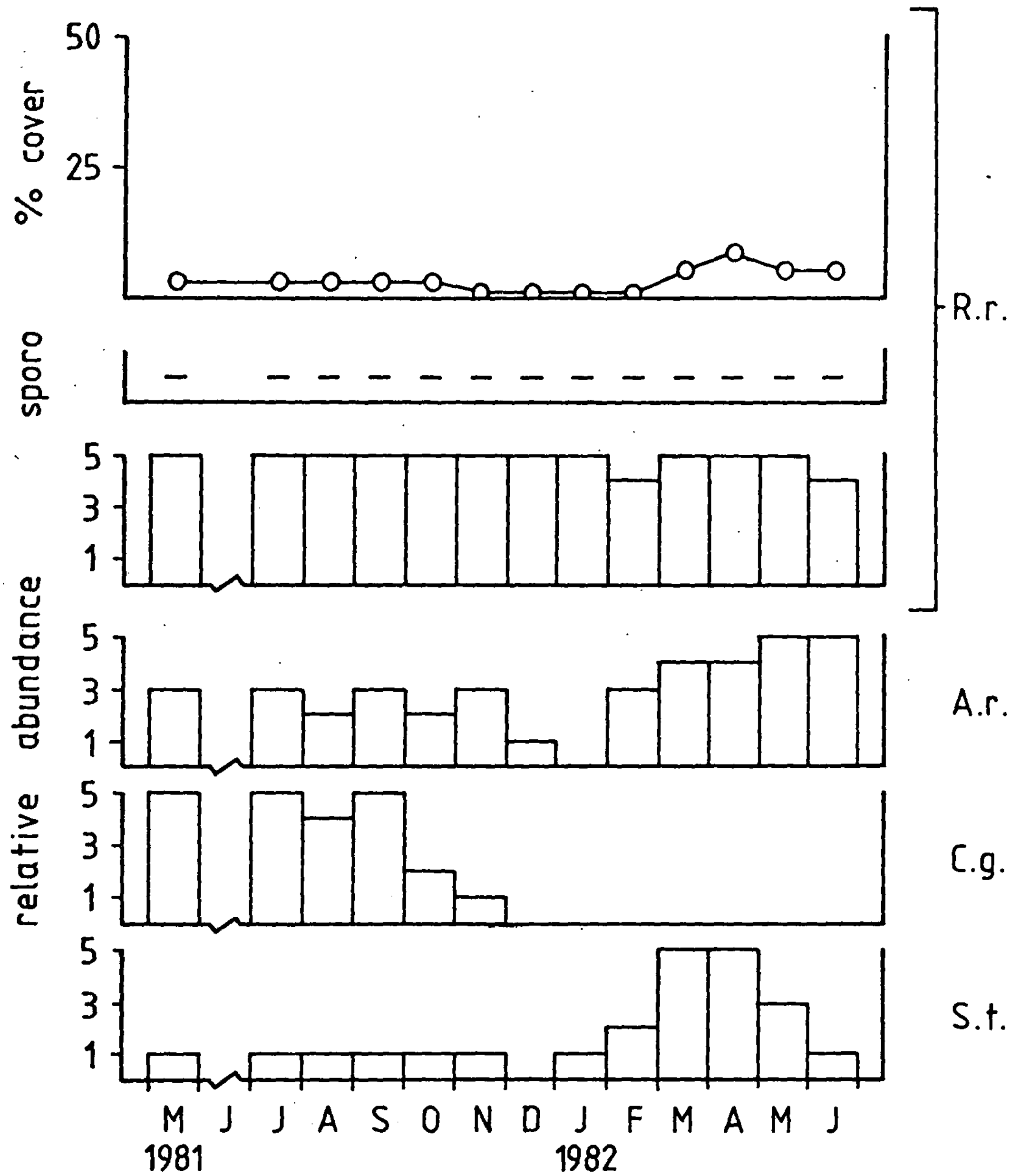
5.32 River Team, Causey Arch (0024-20)

The relative abundance of two aquatic mosses and two macrophytic algae in this stream are shown in Fig. 5.21. As in the upper reach, Rhynchostegium was usually the most abundant macrophyte. Some of the streambed consisted of small stones and gravel, and macrophyte cover (on more stable substrates) was usually between 2.5 and 5.0%. The same species recorded at this site were found upstream, although the relative importance of Cladophora during the first half of the survey was greater in this reach. Unlike reach 0024-05, this alga did not return in spring 1982 after the winter decline. Instead, Stigeoclonium increased to a point where in some months its biomass exceeded that of Rhynchostegium. No sporophytes were ever observed on this population. Amblystegium riparium, which was also sampled for metal analysis, declined in abundance in winter and was present in insufficient quantities during December and January to be sampled. Amblystegium,

Table 5.10. Temporal correlations between environmental variables and enrichment ratios for Zn, Cd, Ba and Pb in <u>Rhynchostegium</u> over one year in the River Team, Kyo Heaugh, reach 0024-05 (n = 13; * = p < 0.05, ** = p < 0.01).

	Zn	Cd	Ba	Pb
temperature	-0.10	0.13	-0.04	-0.20
O.D. 240 nm	-0.08	0.09	0.31	0.26
pH	-0.23	0.29	-0.01	0.06
total alk	-0.18	0.16	-0.31	-0.62 *
filt reac PO4	-0.02	-0.15	0.10	0.06
filt org PO4	-0.02	-0.20	0.06	0.06
Si	-0.17	0.03	-0.06	-0.22
Cl	-0.03	-0.21	0.04	-0.04
Na wat	0.46	-0.27	0.01	-0.28
Mg wat	0.12	0.09	-0.10	-0.57 *
Ca wat	-0.15	-0.03	-0.35	-0.74 **
Mn wat	-0.26	0.62 *	0.54	-0.16
Fe wat	0.12	0.20	0.76 **	0.51
Mg moss	0.08	-0.28	-0.01	-0.12
K moss	0.02	-0.32	-0.29	0.19
Ca moss	0.15	0.05	0.40	0.02
Mn moss	0.45	0.63 *	0.79 **	0.18
Fe moss	0.43	-0.08	-0.18	-0.06

Figure 5.21. Seasonal changes in the relative abundance (1 - 5) of aquatic bryophytes and macroalgae, and the percent cover and sporophyte production of *Rhynchostegium* in the River Team, Causey Arch (R.r. = *Rhynchostegium riparioides*, A.r. = *Amblystegium riparium*, C.g. = *Cladophora glomerata*, S.t. = *Stigeoclonium tenue*).


50
25
% cover
sporo
5
3
1
relative abundance
R.r.
A.r.
C.g.
S.t.
M J J A S O N D J F M A M J
1981
1982

unlike Rhynchostegium, had a marked seasonal variation in abundance over the year.

Concentrations of anions were typically quite high (Fig. 5.22), with filtrable reactive phosphate usually $\geq$ 1.0 mg l^{-1}. All physicochemical variables were erratic seasonally. Extreme peaks in concentrations were observed, but they did not coincide. A pH maximum of 9.8 was recorded in September 1981, but total alkalinity peaked in March. Filtrable reactive phosphorus was greatest in August (5.1 mg l^{-1}) and chloride reached 355 mg l^{-1} in January. Chloride was higher on that month than in any stream previously sampled for Rhynchostegium (section 4.22). Regular seasonal change, with the exception of water temperature, was apparently lacking altogether in these variables.

Some evidence of a seasonal pattern in aqueous magnesium and calcium can be seen (Fig. 5.23), with a steady drop during the autumn and winter. Sodium, manganese and iron concentrations were apparently non-seasonal, but there were definite peaks in all three during January, when chloride reached its maximum.

The most obvious feature of the changes in heavy metals was a twentyfold increase in zinc measured in stream water and both moss species (Fig. 5.24). During the year a gradual shutdown of a local colliery resulted in the lessening of pumped groundwater into the River Team about 2 km upstream of this reach. Concentrations of metals increased markedly simply through a lack of dilution. A close correspondence between zinc in the water and the two mosses was found over the year. Aqueous zinc concentrations reached a peak in April 1982 (T = 7.7, F = 6.9 mg l^{-1}), well above the maximium observed during the intensive survey of Rhynchostegium (section 4.22). Although aqueous zinc declined after the April maximum, the "low" of about 2 mg l^{-1} in

Figure 5.22. Seasonal changes in temperature, optical density, pH, total alkalinty, phosphate (filtrable organic & reactive), chloride and silica in the River Team, Causey Arch.

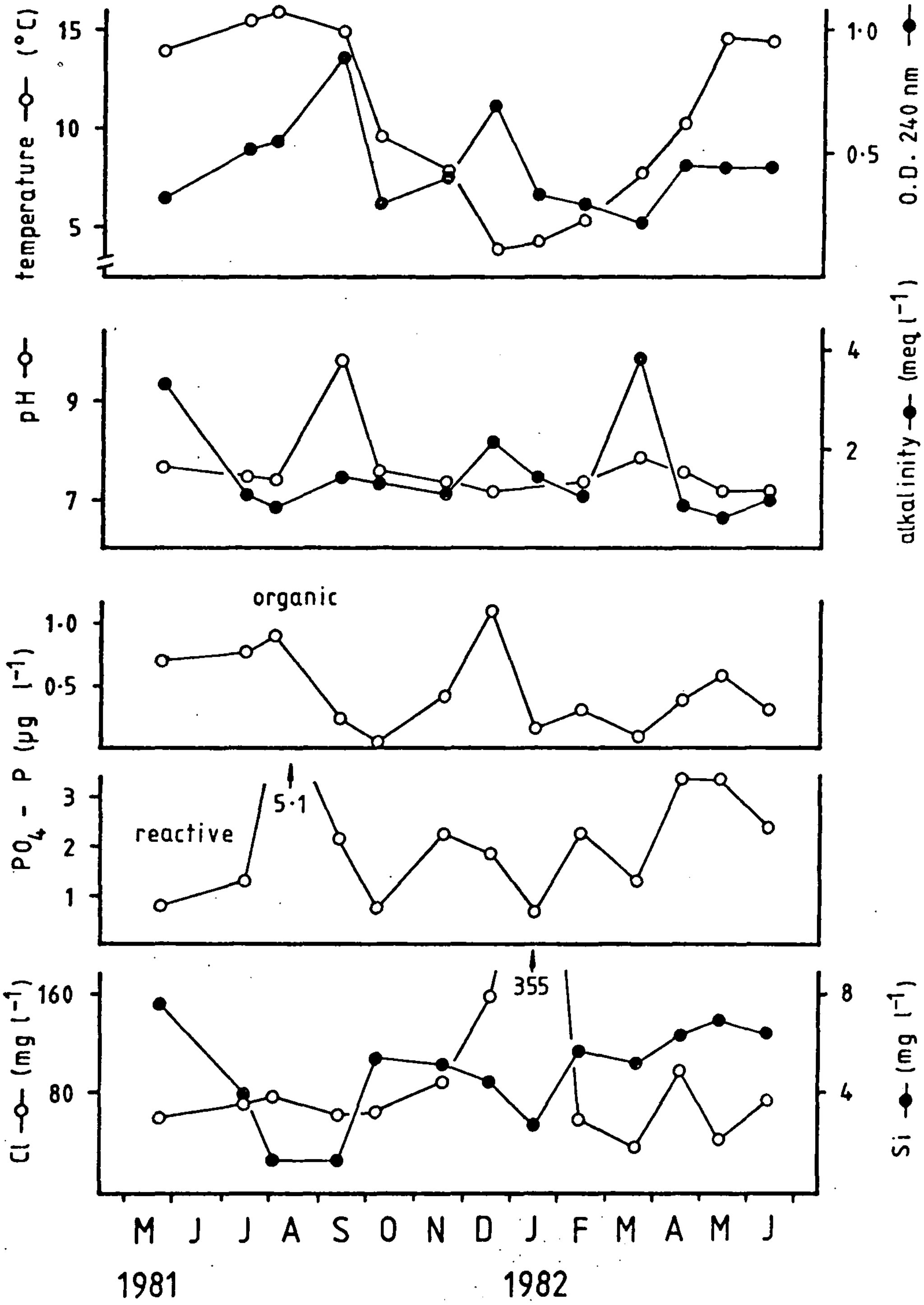

temperature (°C)
O.D. 240 nm
pH
alkalinity (meq l^{-1})
organic
reactive
PO_4 - P (µg l^{-1})
5·1
355
Cl (mg l^{-1})
Si (mg l^{-1})
M J J A S O N D J F M A M J
1981
1982

Figure 5.23. Seasonal changes in aqueous Na, Mg, Ca, Mn and Fe (mg l^{-1}) in the River Team, Causey Arch.

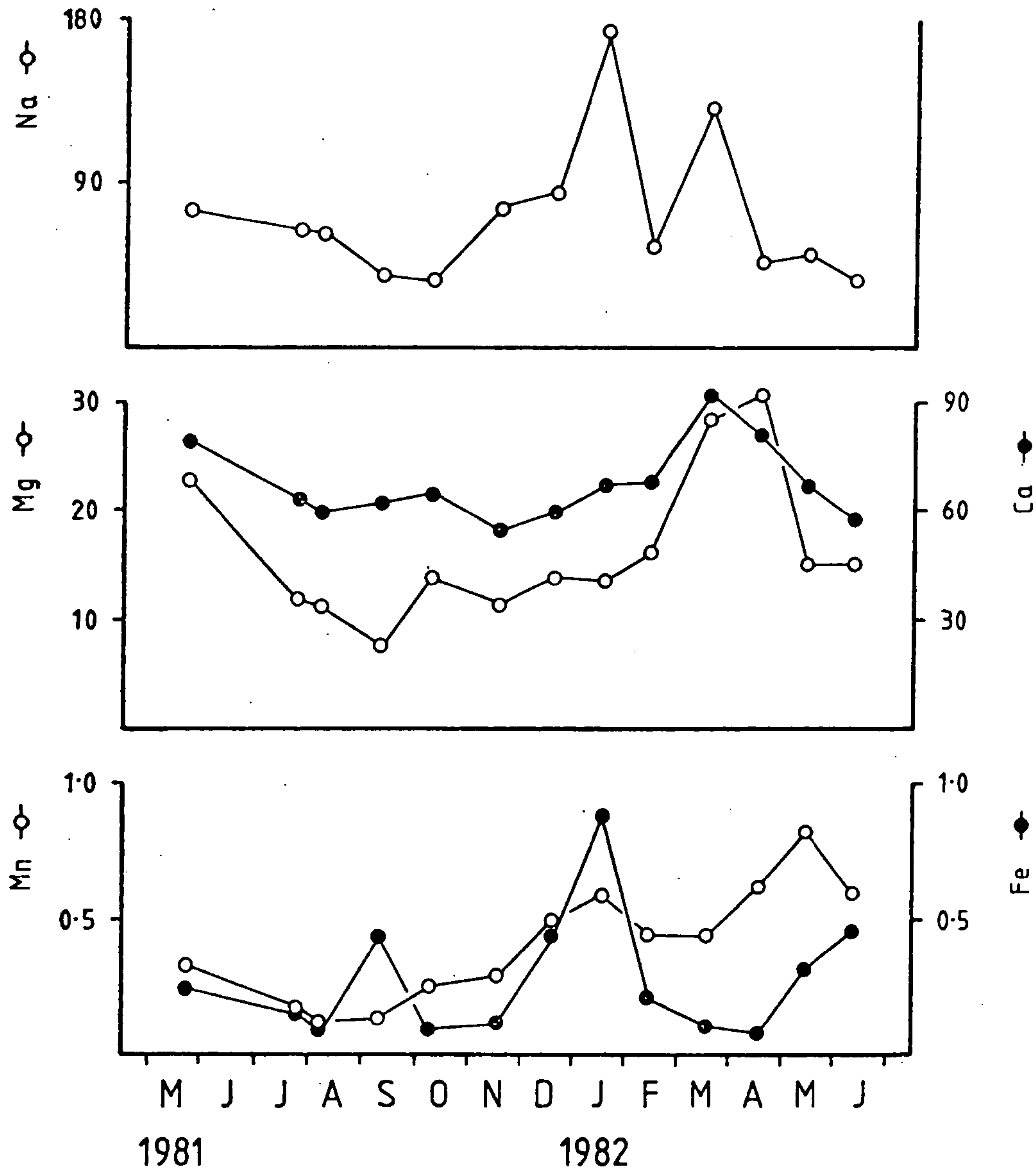
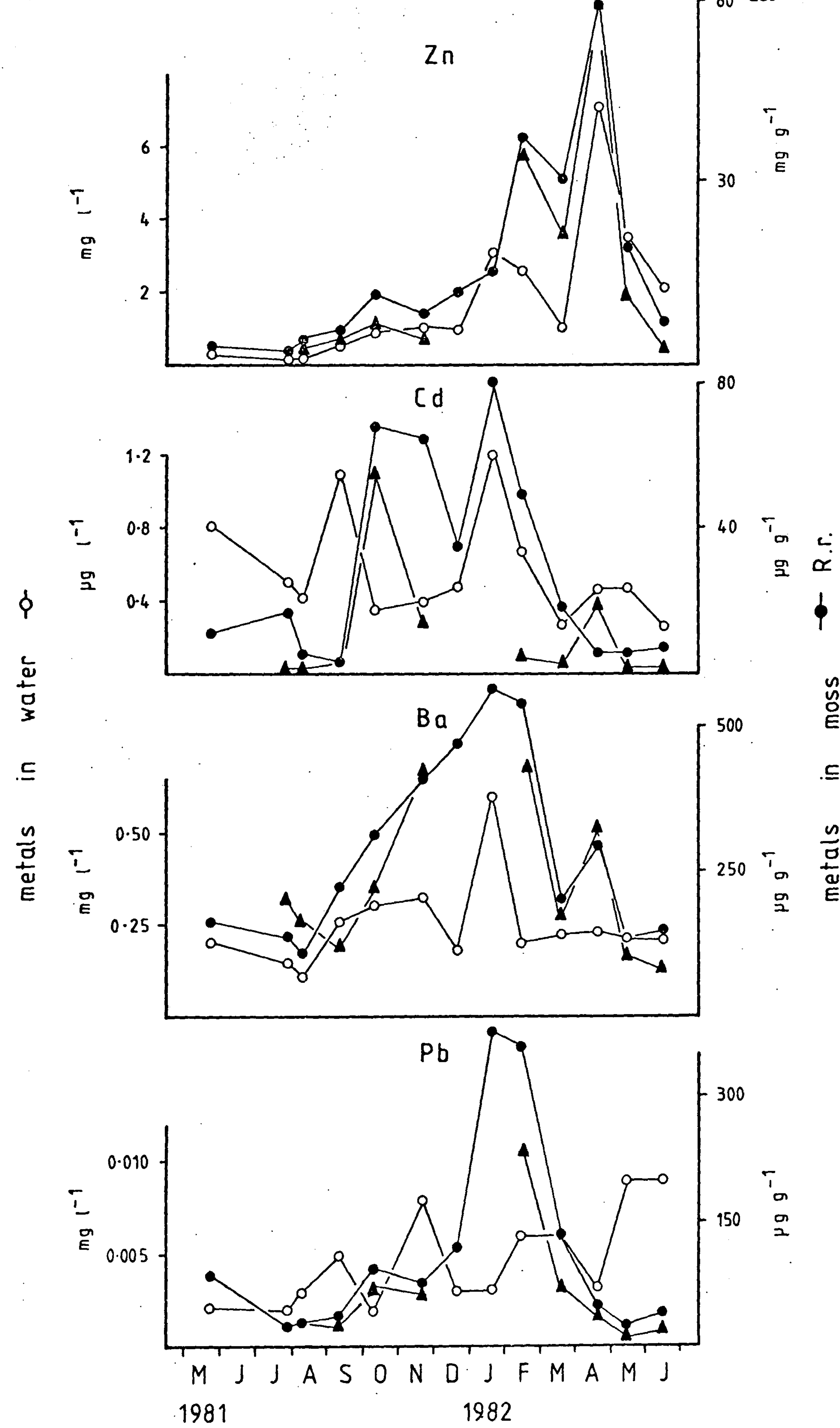

180
90
Na
30
20
10
Mg
90
60
30
Ca
1·0
0·5
Mn
1·0
0·5
Fe
M J J A S O N D J F M A M J
1981
1982

Figure 5.24. Seasonal changes in Zn, Cd, Ba and Pb in stream water and mosses in the River Team, Causey Arch.


Zn
Cd
Ba
Pb
metals in water
metals in moss R.r.
mg l⁻¹
mg g⁻¹
µg l⁻¹
µg g⁻¹
2
4
6
30
60
0·4
0·8
1·2
40
80
0·25
0·50
250
500
0·005
0·010
150
300
M J J A S O N D J F M A M J
1981
1982

June was nearly ten times higher than was measured the previous year. Cadmium, barium and lead also increased but all peaked in January- and February, rather than April. Unlike zinc, these metals returned to similar concentrations to the previous year. On average, accumulation of all four metals was greater in Rhynchostegium than Amblystegium.

Concentrations of cadmium, barium and lead in both moss species followed fairly closely the changes in aqueous concentrations over the year, although less precisely than for zinc. Correlations for Amblystegium and Rhynchostegium (Table 5.11) indicate strong

Table 5.11. Temporal correlations between metals accumulated by Amblystegium riparium and Rhynchostegium riparioides with aqueous metals over one year in the River Team, Causey Arch, reach 0024-20 (A.r.: n = 10, R.r.: n = 13; * = p < 0.05, ** = p < 0.01, *** = p < 0.001).

	Amblystegium		Rhynchostegium	
	T	F	T	F
Zn	0.87***	0.90***	0.85***	0.86*
Cd	-0.13	-0.26	-0.01	0.19
Ba	0.36	0.49	0.17	0.64*
Pb	0.09	0.01	0.33	-0.11

correlations for zinc accumulation by both species. One other significant correlation was for barium accumulation by Rhynchostegium. While it appears there was a fairly close correspondence in metal concentrations between the two species over the year, neither correlated significantly with seasonal fluctuations in cadmium or lead in stream water.

Seasonal variations in enrichment ratios for zinc and cadmium in Amblystegium corresponded with fluctuations in several environmental factors (Table 5.12). Unlike nearly all other populations (this chapter) and previous results (chapter 4), aqueous calcium and total alkalinity (TA) correlated positively with greater accumulation of zinc and lead. Further, no variables were signficantly correlated with accumulation of cadmium or barium. These apparently atypical results were also found for Rhynchostegium (Table 5.13). Although other variables also correlated significantly (negative) with lead accumulation, the relationships for aqueous calcium and TA were again the strongest.

5.33 River Wear, Shincliffe (0008-65)

There was a diverse flora in this reach on the Wear (Fig. 5.25). Unlike the other sites studied, Rhynchostegium was not the dominant macrophyte at any time during the year. Its cover was always low and sporophytes were never observed in this population (sporophyte production has been observed in populations of this species further upstream in Weardale, however). Fontinalis antipyretica was usually the most abundant, although its relative abundance was exceeded in the spring and summer by a massive growth of the alga Cladophora glomerata. Cladophora was found during all the months sampled, unlike both populations in the River Team. During the autumn and winter Fissidens crassipes formed a short, dense turf over much of the river-bed, when most of the larger plants (e.g. Fontinalis and several angiosperm species) were stripped back during high flows. In contrast, Enteromorpha flexuosa was common only during periods of reduced flow,

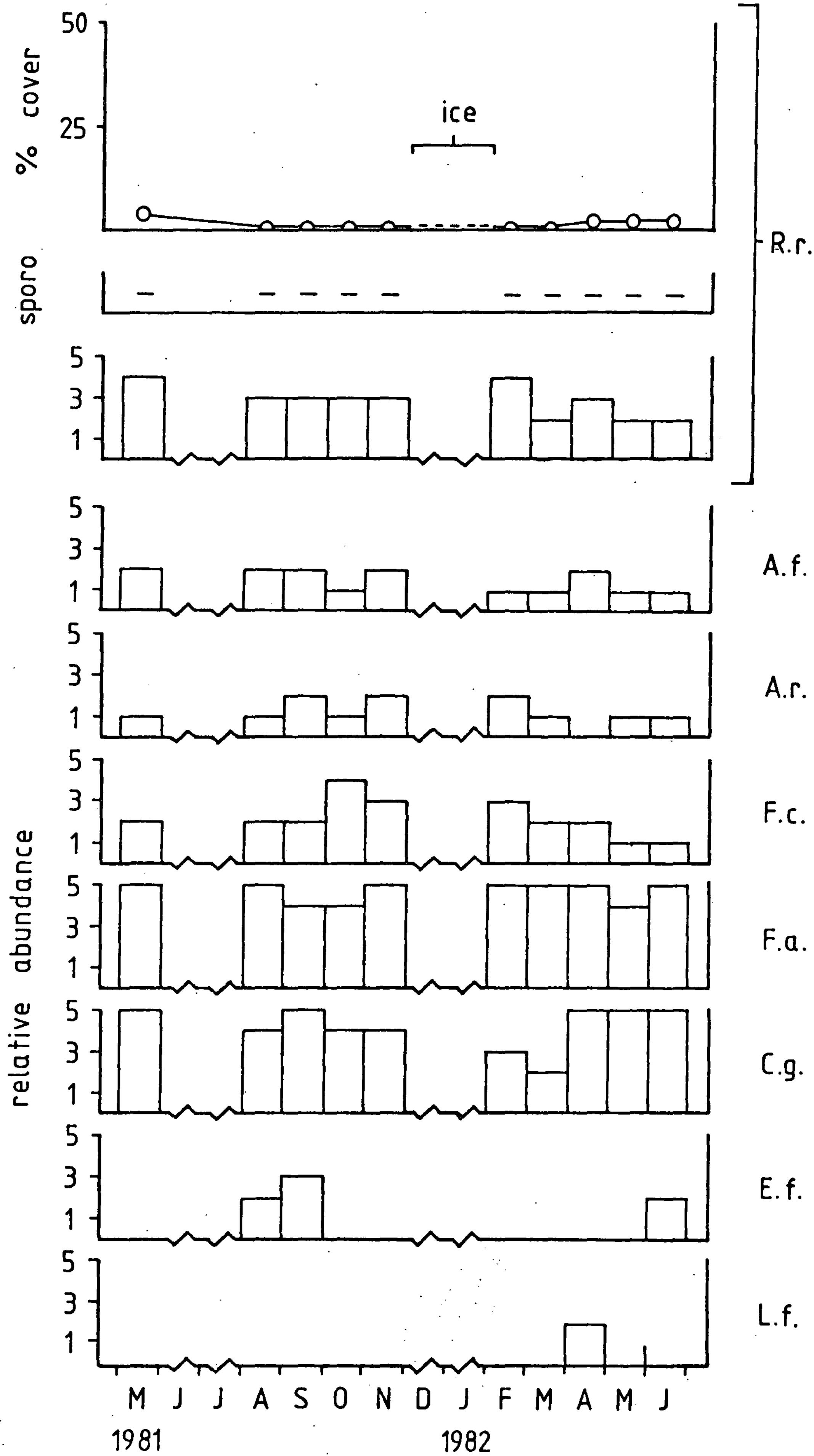
Table 5.12. Temporal correlations between environmental variables and enrichment ratios for Zn, Cd, Ba and Pb in Amblystegium riparium over one year in the River Team, Causey Arch, reach 0024-20 ($n = 10$; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

	Zn	Cd	Ba	Pb
temperature	-0.52	-0.36	-0.41	-0.64 *
O.D. 240 nm	-0.54	-0.36	-0.33	-0.65 *
pH	0.06	-0.11	-0.42	0.01
total alk	0.89 ***	-0.01	-0.26	0.86 **
filt reac PO4	-0.36	-0.44	0.20	-0.50
filt org PO4	-0.41	-0.50	0.34	0.59
Si	0.03	0.24	0.06	0.16
Cl	-0.48	0.14	0.43	-0.50
Na wat	0.83 **	-0.30	0.05	0.68 *
Mg wat	0.58	0.04	0.21	0.55
Ca wat	0.82 **	-0.01	0.06	0.77 **
Mn wat	-0.08	-0.12	-0.08	-0.03
Fe wat	-0.51	0.40	-0.54	-0.45
Mg moss	0.24	0.46	0.41	0.31
K moss	-0.60	0.25	-0.30	-0.53
Ca moss	0.24	0.21	0.35	0.25
Mn moss	-0.17	-0.14	0.25	-0.30
Fe moss	0.37	0.61	0.50	0.54

Table 5.13. Temporal correlations between environmental variables and enrichment ratios for Zn, Cd, Ba and Pb in Rhynchostegium over one year in the River Team, Causey Arch, reach 0024-20 (n = 13; * = p < 0.05, ** = p < 0.01, *** = p < 0.001).

	Zn	Cd	Ba	Pb
temperature	-0.24	-0.52	-0.66 *	-0.64 *
O.D. 240 nm	-0.31	-0.35	0.05	-0.56 *
pH	0.16	-0.22	-0.65 *	0.52
total alk	0.70 **	0.01	-0.07	0.62 *
filt reac PO4	0.23	-0.41	0.02	-0.56 *
filt org PO4	-0.18	-0.33	0.28	-0.44
Si	0.02	0.10	0.08	-0.03
Cl	-0.32	0.09	0.11	0.48
Na wat	0.29	0.04	-0.06	0.83 ***
Mg wat	0.43	-0.20	-0.03	0.36
Ca wat	0.60 *	-0.24	-0.17	0.59 *
Mn wat	-0.17	-0.18	0.11	0.17
Fe wat	-0.42	-0.19	-0.01	0.31
Mg moss	0.23	0.48	0.32	0.24
K moss	-0.32	0.53	-0.18	-0.44
Ca moss	0.14	0.10	0.50	0.37
Mn moss	-0.07	0.40	0.67 *	0.01
Fe moss	0.06	0.52	0.70 **	0.53

Figure 5.25. Seasonal changes in the relative abundance (1 - 5) of aquatic bryophytes and macroalgae, and the percent cover and sporophyte production of _Rhynchostegium_ in the River Wear, Shincliffe (R.r. = _Rhynchostegium riparioides_, A.f. = _Amblystegium fluviatile_, A.r. = _Amblystegium riparium_, F.c. = _Fissidens crassipes_, F.a. = _Fontinalis antipyretica_, C.g. = _Cladophora glomerata_, E.f. = _Enteromorpha flexuosa_, L.f. = _Lemanea fluviatilis_).


50
25
% cover
ice
sporo
R.r.
5
3
1
A.f.
A.r.
F.c.
F.a.
C.g.
E.f.
L.f.
relative abundance
M J J A S O N D J F M A M J
1981
1982

with many of the detached plants floating on the water surface in pools.

Seasonal trends in physicochemical variables were clearly evident (Fig. 5.26). Temperature extremes were probably underestimated, as between December and January the river was covered with ice and not sampled. Total alkalinity and pH values indicate that the river remained relatively alkaline throughout the year, although somewhat depressed during the autumn-winter. Anions (FRP, ORP, Cl, Si) were in fairly high concentrations over the year and followed a similar seasonal pattern, with maxima in summer and minima in winter and spring.

The highest concentrations of aqueous sodium, magnesium and calcium for any site during the seasonal surveys were measured here (Fig. 5.27). These elements had a distinct seasonal pattern, much like that observed for the anions. Total alkalinity reached a maximum in August when filtrable calcium reached 108 mg l^{-1}. The September peak in chloride (155 mg l^{-1}) coincided with the maximum filtrable sodium measured (F = 188 mg l^{-1}). Manganese and iron were not, however markedly seasonal in their variation.

Concentrations of aqueous zinc, cadmium and lead were amongst the lowest measured during the seasonal surveys, although barium was higher than most (Fig. 5.28). The seasonal patterns in the variation of zinc and lead concentrations were similar, with maximum levels during the winter-spring period. Barium increased somewhat earlier in the year, while cadmium levels were near detection limits (erratic plot a function of low precision in this range) and difficult to interpret. Concentrations of metals in both mosses corresponded fairly closely with aqueous metals. Correlations (Table 5.14) indicate these relations

Figure 5.26. Seasonal changes in temperature, optical density, pH, total alkalinty, phosphate (filtrable organic & reactive), chloride and silica in the River Wear, Shincliffe.

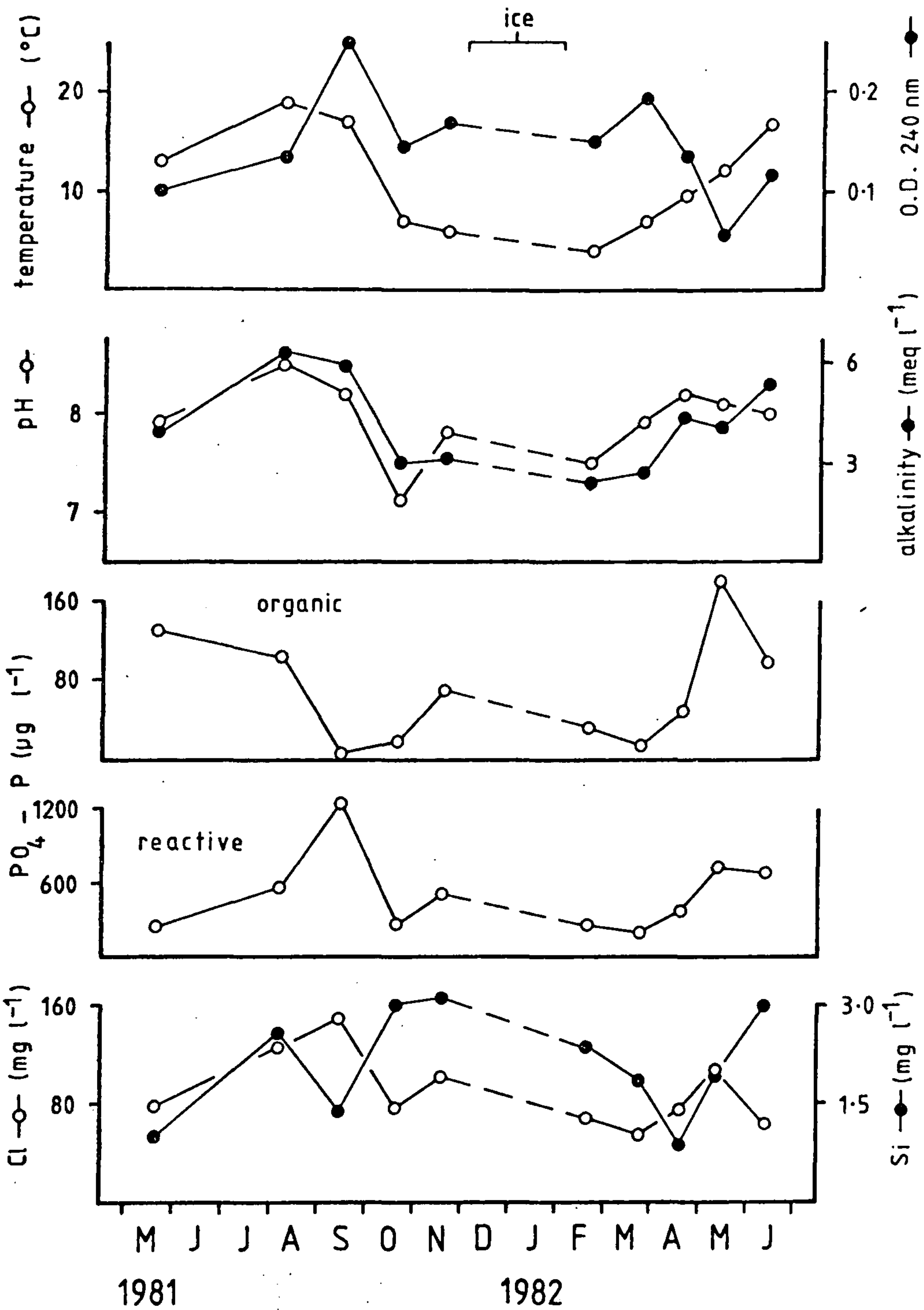

ice
temperature (°C)
20
10
O.D. 240 nm
0·2
0·1
pH
8
7
alkalinity (meq l⁻¹)
6
3
160
80
organic
PO4 - P (µg l⁻¹)
1200
600
reactive
Cl (mg l⁻¹)
160
80
Si (mg l⁻¹)
3·0
1·5
M J J A S O N D J F M A M J
1981
1982

Figure 5.27. Seasonal changes in aqueous Na, Mg, Ca, Mn and Fe (mg l^{-1}) in the River Wear, Shincliffe.

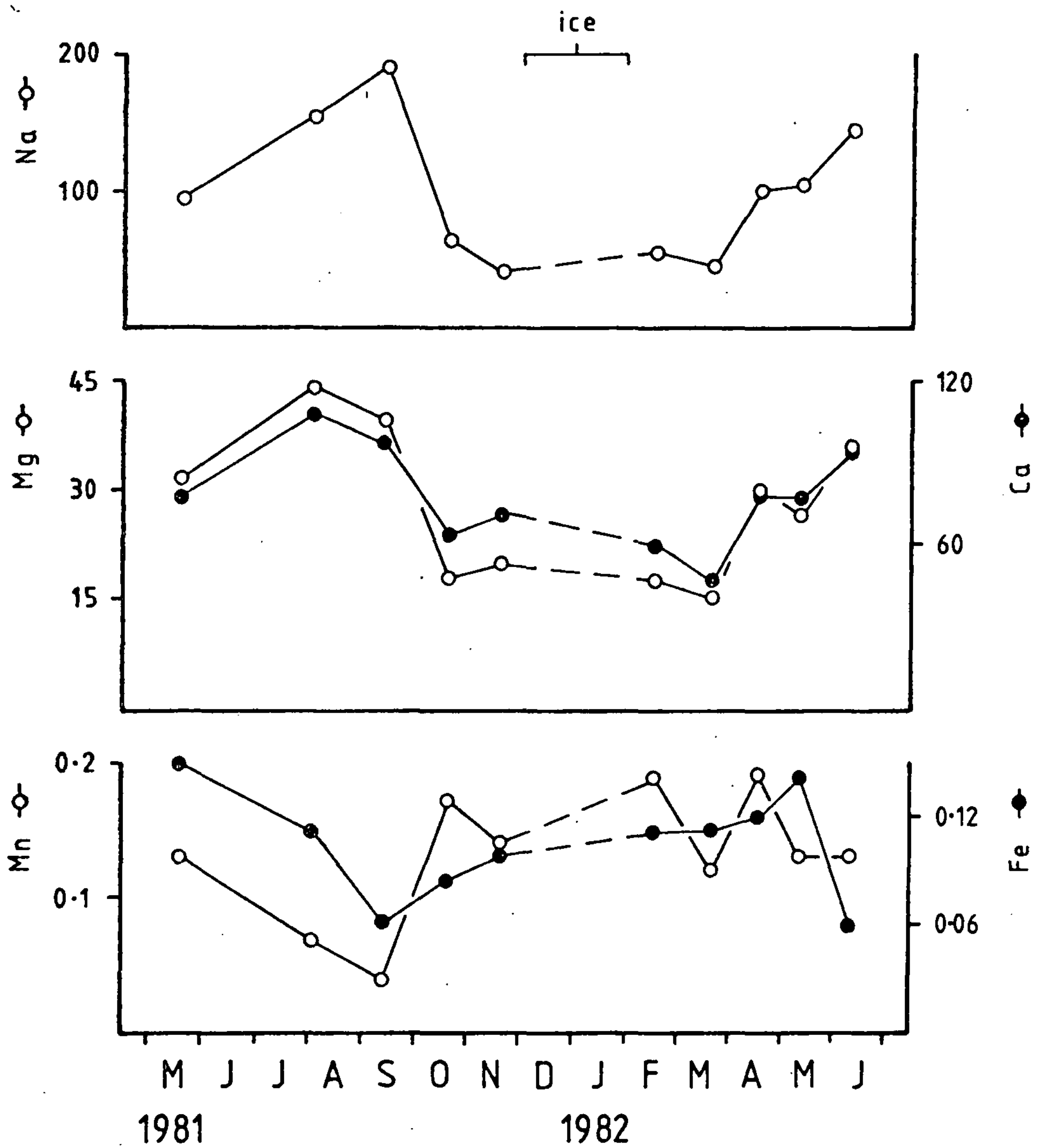

ice
Na
200
100
Mg
45
30
15
120
60
Ca
Mn
0·2
0·1
0·12
0·06
Fe
M J J A S O N D J F M A M J
1981
1982

Figure 5.28. Seasonal changes in Zn, Cd, Ba and Pb in stream water and mosses in the River Wear, Shincliffe.

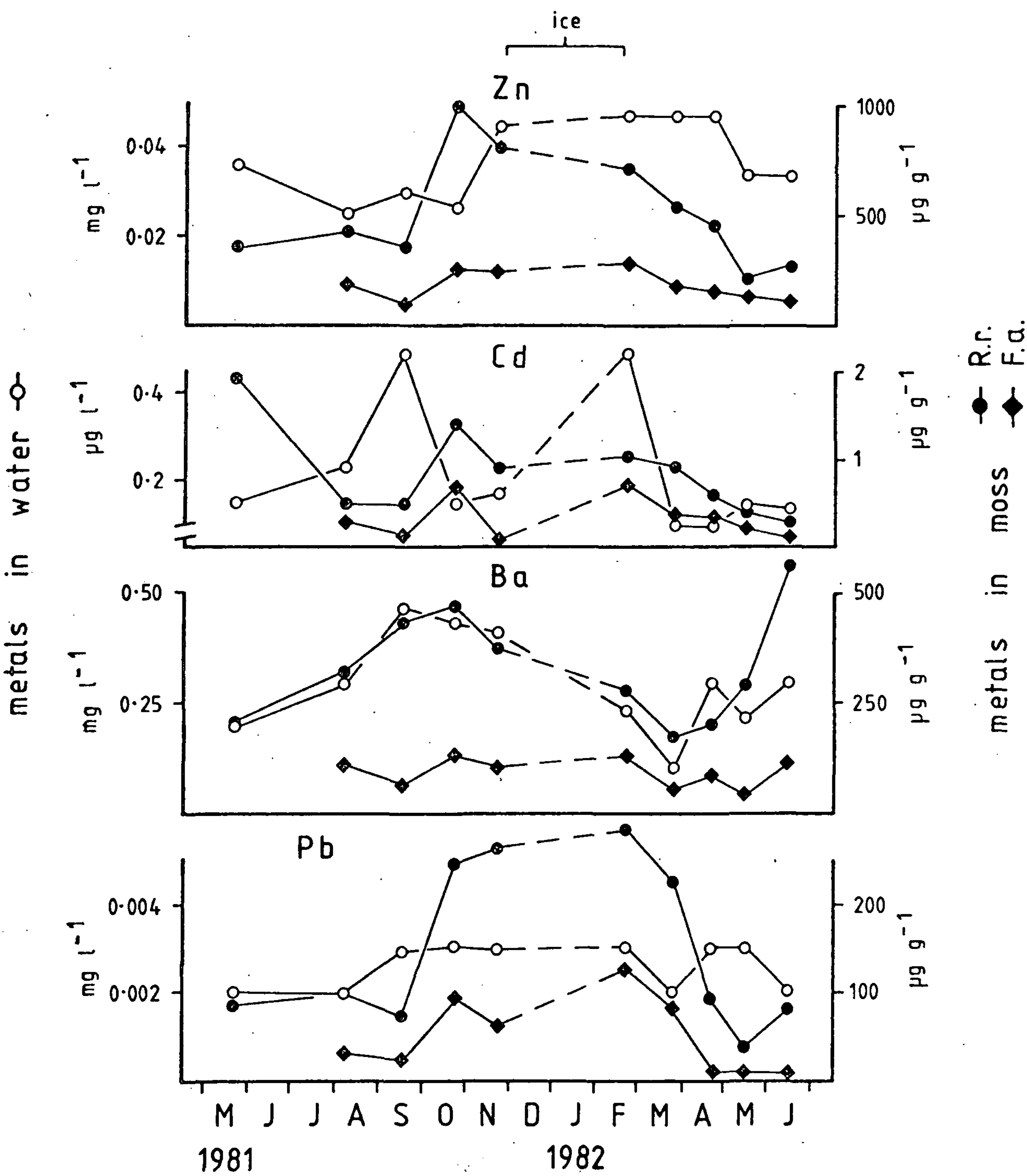

ice
Zn
Cd
Ba
Pb
metals in water
metals in moss
R.r.
F.a.
mg l⁻¹
µg l⁻¹
µg g⁻¹
0·04
0·02
1000
500
0·4
0·2
2
1
0·50
0·25
500
250
0·004
0·002
200
100
M J J A S O N D J F M A M J
1981
1982

Table 5.14. Temporal correlations between metals accumulated by Fontinalis antipyretica and Rhynchostegium riparioides with aqueous aqueous metals over one year in the River Wear, Shincliffe, reach 0008-65 (F.a.: n = 9, R.r.: n = 10; * = $p < 0.05$).

	Fontinalis		Rhynchostegium	
	T	F	T	F
Zn	0.49	0.36	0.19	0.12
Cd	0.35	0.11	0.03	-0.09
Ba	0.53	0.45	0.19	0.64*
Pb	0.47	0.14	0.41	0.26

were significant only for barium in Rhynchostegium. Apical tips of this species accumulated metals in roughly four times the concentrations measured in Fontinalis year round. Seasonal trends in accumulation by the two species were quite similar.

Correlations between monthly enrichment ratios for Fontinalis and seasonal changes in physical and chemical variables (Table 5.15) indicate that the most pronounced effect was on lead accumulation. As many as five factors were negatively correlated ($p < 0.05$), all of which were in low levels during the peak in lead accumlation. Cadmium and barium enrichment ratios were also negatively correlated with filtrable reactive phosphate concentrations. The analysis identified several similar variables relating to lead accumulation by Rhynchostegium (Table 5.16); seven factors were negatively correlated. Lead accumulation was also positively correlated with greater iron in the moss.

Table 5.15. Temporal correlations between environmental variables and enrichment ratios for Zn, Cd, Ba and Pb in *Fontinalis antipyretica* over one year in the River Wear, Shincliffe, reach 0008-65 ($n = 9$; * = $p < 0.05$).

	Zn	Cd	Ba	Pb
temperature	-0.29	-0.43	-0.42	-0.66
O.D. 240 nm	-0.18	-0.21	-0.01	0.20
pH	-0.58	-0.45	-0.27	-0.63
total alk	-0.28	-0.53	-0.52	-0.73 *
filt reac PO4	-0.48	-0.68 *	-0.71 *	-0.69 *
filt org PO4	-0.10	-0.14	-0.10	-0.46
Si	0.56	0.07	0.23	0.23
Cl	-0.06	-0.63	-0.67 *	-0.46
Na wat	-0.38	-0.52	-0.52	-0.70 *
Mg wat	-0.27	-0.56	-0.47	-0.71 *
Ca wat	-0.18	-0.64	-0.56	-0.76 *
Mn wat	0.26	0.54	0.39	0.36
Fe wat	0.04	0.20	0.25	0.11
Mg moss	0.48	-0.04	0.21	0.54
K moss	0.47	0.21	-0.07	0.28
Ca moss	0.42	-0.32	0.04	0.28
Mn moss	-0.01	-0.29	-0.32	-0.46
Fe moss	0.25	-0.18	0.58	0.63

Table 5.16. Temporal correlations between environmental variables and enrichment ratios for Zn, Cd, Ba and Pb in Rhynchostegium over one year in the River Wear, Shincliffe, reach 0008-65 (n = 10; * = p < 0.05, ** = p < 0.01).

	Zn	Cd	Ba	Pb
temperature	-0.33	-0.04	0.20	-0.72 *
O.D. 240 nm	0.11	-0.19	0.01	0.28
pH	-0.68 *	-0.45	-0.08	-0.66 *
total alk	-0.28	-0.49	0.01	-0.74 *
filt reac PO4	-0.28	-0.69 *	-0.01	-0.64 *
filt org PO4	-0.39	0.01	0.21	-0.45
Si	0.50	-0.25	0.53	0.37
Cl	-0.01	-0.50	-0.43	-0.49
Na wat	-0.31	-0.58	0.08	-0.76 **
Mg wat	-0.32	-0.44	-0.03	-0.74 *
Ca wat	-0.22	-0.54	-0.05	-0.72 *
Mn wat	0.27	0.29	-0.10	0.42
Fe wat	-0.30	0.39	-0.27	-0.07
Mg moss	0.35	-0.32	-0.47	0.20
K moss	0.16	-0.24	-0.48	-0.14
Ca moss	0.58	-0.26	-0.15	0.35
Mn moss	0.20	-0.36	0.27	-0.34
Fe moss	0.12	0.07	-0.06	0.82 **

5.4 Time series analysis of metal accumulation

In this section, time series analyses using a cross-correlation are applied to the metal accumulation data for the three aquatic mosses studied. The possibility that strictly temporal effects may influence metal accumulation, in contrast to physical or chemical factors, is examined. The test was whether in some populations, there was evidence of a consistent delay between changes in metal concentrations in stream water and in the mosses. In each, cross-correlations between these two components were calculated using increasing monthly lags, from zero to three months. For example, if variations in cadmium concentrations in one species were to follow a similar, but staggered seasonal pattern to aqueous concentrations, significant, positive cross-correlations will be observed for a particular lag period.

5.41 "High Crag Burn" (0101-05)

Seasonal trends in zinc accumulation were found to be significantly correlated (section 5.21) and increasing lags did not produce stronger correlations for this element (Table 5.17). Only total barium correlated temporally with barium accumulation over the year. Filtrable barium differed from this, where a somewhat stronger (but non-significant) relation was found at the two-month lag. The plot of the seasonal changes for lead (Fig. 5.04) suggested a lag and at a three month lag this relationship was stronger, but insignificant.

5.42 Lee Springs (0289-98)

In Lee Springs, total and/or filtrable zinc, barium and lead correlated significantly without any apparent lag effect (Table 5.18).

Table 5.17. Cross-correlations computed between metals accumulated by Rhynchostegium and aqueous metals in "High Crag Burn," reach 0101-05, over one year for three increasing monthly lags (maximum positive correlations are underlined; * = p < 0.05).

	0 month		1 month		2 month		3 month	
	T	F	T	F	T	F	T	F
Zn	0.72*	0.65*	0.31	0.37	0.22	0.22	-0.24	-0.29
Cd	0.30	0.24	0.08	0.15	-0.18	-0.18	-0.33	-0.36
Ba	0.57*	0.14	0.11	0.22	0.33	0.48	0.27	0.33
Pb	-0.09	-0.43	-0.23	-0.29	-0.23	0.07	-0.06	0.28

Table 5.18. Cross-correlations computed between metals accumulated by Rhynchostegium and aqueous metals in Lee Springs, reach 0289-98 over one year for three increasing monthly lags (maximum positive correlations are underlined; * = p < 0.05, ** = p < 0.01, *** = p < 0.001).

	0 month		1 month		2 month		3 month	
	T	F	T	F	T	F	T	F
Zn	0.62*	0.64*	-0.04	0.08	0.05	-0.08	-0.18	-0.22
Cd	-0.10	0.08	0.01	-0.05	0.30	0.85***	0.09	0.14
Ba	0.60*	0.44	0.48	0.30	0.15	0.10	0.33	0.30
Pb	0.66*	0.33	0.26	0.49	0.03	-0.03	-0.02	0.02

No obvious seasonal relation was seen between cadmium in moss and water (Fig. 5.08), but on a two month lag the filtrable fraction correlated highly significantly ($p < 0.001$). Otherwise, the analysis indicated no lag effects for metal accumulation.

5.43 River West Allen (0085-50)

Zinc accumulation by Rhynchostegium followed a similar seasonal trend to changes in aqueous concentrations (Fig. 5.12), and cross-correlations indicate this was the closest temporal relationship (Table 5.19). None of the lags tested identified any further significant relationships in metal accumulation. Hence it may be assumed that no strictly temporal effects were invloved.

5.44 "Race Fell Burn" (0310-90)

While significant temporal correlations were found for accumulation of cadmium, barium and lead by this Rhynchostegium population, zinc accumulation was perhaps staggered (section 5.24). Cross-correlations (Table 5.20) support these earlier findings and reveal a significant correlation ($p < 0.05$) for zinc accumulation when lagged one month after the temporal pattern for aqueous zinc.

5.45 River Team, Kyo Heaugh (0024-05)

The time-series analysis for zinc accumulation by Rhynchostegium at this site (Table 5.21) reveals that the correlation with aqueous zinc had no apparent lag effect. No other significant relationships were found by this analysis for the other elements. One major change was found after a one month lag with barium accumulation, whose correlation coefficient changed from -0.64 to +0.41.

Table 5.19. Cross-correlations computed between metals accumulated by Rhynchostegium and aqueous metals in the River West Allen reach 0085-50, over one year for three increasing monthly lags (maximum positive correlations are underlined; * = $p < 0.05$).

	0 month		1 month		2 month		3 month	
	T	F	T	F	T	F	T	F
Zn	0.61*	0.56	0.24	0.16	-0.14	-0.16	0.12	0.12
Cd	0.33	0.32	0.31	0.32	0.01	-0.01	0.14	0.14
Ba	-0.48	-0.39	-0.32	-0.34	-0.44	-0.44	-0.16	-0.15
Pb	-0.36	-0.29	-0.24	-0.16	-0.15	-0.10	-0.19	-0.26

Table 5.20. Cross-correlations computed between metals accumulated by Rhynchostegium and aqueous metals in "Race Fell Burn," reach 0310-90, over one year for three increasing monthly lags (maximum positive correlations are underlined; * = $p < 0.05$, ** = $p < 0.01$).

	0 month		1 month		2 month		3 month	
	T	F	T	F	T	F	T	F
Zn	0.19	0.44	0.70*	0.71*	-0.19	0.01	-0.39	-0.38
Cd	0.62*	0.76**	-0.15	-0.03	-0.19	-0.22	0.47	0.23
Ba	0.56	0.63*	0.66*	0.47	0.32	0.25	-0.43	-0.48
Pb	0.30	0.52	-0.28	0.32	-0.46	0.06	-0.25	-0.10

Table 5.21. Cross-correlations computed between metals accumulated by Rhynchostegium and aqueous metals in the River Team, Kyo Heaugh, reach 0024-05, over one year for three increasing monthly lags (maximum positive correlations are underlined; * = p < 0.05, ** = p < 0.01).

	0 month		1 month		2 month		3 month	
	T	F	T	F	T	F	T	F
Zn	0.54	0.72**	0.62	0.52	0.22	0.14	0.12	0.04
Cd	-0.25	0.15	0.10	0.11	0.07	-0.05	0.16	-0.17
Ba	-0.67*	-0.64*	-0.21	0.41	-0.14	-0.55	0.01	-0.28
Pb	-0.03	0.18	0.18	-0.06	0.33	0.41	0.54	-0.12

5.46 River Team, Causey Arch (0024-20)

During the period of increasing zinc pollution in the Team, accumulation of this metal by both species was highly correlated with aqueous concentrations (Table 5.11). There was also no evidence of any delay in response in the time-series analysis (Table 5.22). For Amblystegium, although no other significant relations were discovered, three of the four metals correlate most strongly without any lag. These observations are in large part true for Rhynchostegium as well, although a greater (but non-significant) relation was found for the accumulation of cadmium at a three month lag.

5.47 River Wear, Shincliffe (0008-65)

At this site, few significant correlations were observed between metals in either Rhynchostegium or Fontinalis and river water (section 5.27). A large number of physical and chemical factors were related, although zinc was apparently influenced only by pH. This metal stands out in the time series analysis with Fontinalis (Table 5.23), in that a clear improvement in the relationship for accumulation was found for a one month lag. Both total and filtrable zinc were significantly ($p < 0.05$) correlated. The relationship for cadmium, while improved at the two month lag, was not significant. Results for Rhynchostegium were quite similar to that for Fontinalis. A significant correlation was also found at a one month lag for zinc accumulation. The correlation for cadmium accumulation at a two month lag was greater, significantly so for the filtrable fraction.

Table 5.22. Cross-correlations between metals accumulated by the mosses Amblystegium riparium and Rhynchostegium riparioides and aqueous metals in the River Team, Causey Arch, reach 0024-20, over one year for three increasing monthly lags (maximum positive correlations are underlined; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

	0 month		1 month		2 month		3 month	
	T	F	T	F	T	F	T	F
Amblystegium								
Zn	0.87***	0.90***	0.38	0.28	0.45	0.45	0.12	0.08
Cd	-0.13	0.25	-0.27	-0.16	-0.10	-0.07	0.08	0.17
Ba	0.36	0.49	-0.01	-0.03	-0.29	0.26	-0.21	-0.28
Pb	0.09	0.01	-0.40	-0.41	0.06	0.17	0.33	0.47
Rhynchostegium								
Zn	0.85***	0.86***	0.48	0.40	0.49	0.47	0.18	0.13
Cd	-0.01	0.19	-0.17	0.01	-0.06	0.17	0.01	0.23
Ba	0.17	0.64*	0.16	0.36	-0.01	0.12	-0.13	-0.14
Pb	0.33	-0.11	-0.11	-0.21	-0.25	-0.35	0.24	0.20

Table 5.23. Cross-correlations between metals accumulated by the mosses Fontinalis antipyretica and Rhynchostegium riparioides and aqueous metals in the River Wear, Shincliffe, reach 0008-65, over one year for three increasing monthly lags (maximum positive correlations are underlined; * = $p < 0.05$).

	0 month		1 month		2 month		3 month	
	T	F	T	F	T	F	T	F
Fontinalis								
Zn	0.49	0.36	0.72*	0.69*	0.38	0.34	-0.11	0.02
Cd	0.35	0.11	-0.44	-0.44	0.50	0.38	-0.23	-0.40
Ba	0.53*	0.45	-0.03	-0.11	-0.08	-0.04	-0.37	-0.26
Pb	0.47	0.14	0.45	-0.12	0.12	0.37	0.29	-0.20
Rhynchostegium								
Zn	0.19	0.12	0.70*	0.69*	0.65	0.54	0.27	0.34
Cd	0.03	-0.09	-0.02	-0.06	0.58	0.69*	-0.42	-0.38
Ba	0.19	0.64*	0.27	0.36	0.05	-0.06	-0.22	-0.35
Pb	0.41	0.26	0.50	0.05	0.28	0.06	0.20	-0.12

5.5 Comparison of results from contrasting sites

As sites for this seasonal survey were chosen to compare metal accumulation in different types of rivers, it is not surprising that seasonal events differed amongst sites, as did the actual measurements themselves. To illustrate these differences, the results from the seven are summarized and compared.

5.51 Biological results

A noticeable difference was found between populations of Rhynchostegium from upland and lowland sites in that sporophytes were produced in abundance and for several months (autumn-winter or winter-spring) in all upland populations, but were never seen on lowland plants. This did not relate to the relative abundance of the species, as it was sparse and abundant in both types of river. The percent cover was apparently more common in smaller streams (the larger upland stream, River West Allen had low cover), although cover varied seasonally in these sites as well.

Other species also varied in seasonal abundance and in some cases trends differed between sites. Cladophora glomerata declined in winter months in the Team (0024-05), but not so in the Wear (0008-65). No bryophyte was independent of the seasonal wax and wane during the year, although as a group they must be considered truly perennial in habit, unlike algal species such as Enteromorpha or Lemanea.

5.52 Water Chemistry

Concentrations of anions and metals (excluding heavy metals) differed markedly between the seven sites, but some major patterns were identified. First, pH and total alkalinity usually declined between October and December, a difference which was most noticeable in larger streams. Phosphorus was commonly greatest during the autumn or early winter, but organic and reactive forms did not always behave similarly. Chloride and sodium concentrations typically dropped during periods of increased flow (autumn and/or winter), although brief pulses were also observed. In one site, "Race Fell Burn" (0310-90), increased chloride was not measured during any winter month; it was also away from any roads. Iron (and sometimes manganese) levels increased in the autumn and winter months along with optical density, particularly in upland streams. Magnesium and calcium in stream water were typically lowest during these periods in all rivers, upland and lowland. The Team at Causey Arch was clearly the most unpredictable river over the year, with few obvious seasonal trends. Abrupt increases of several variables appeared on different months, for example: pH in September, total alkalinity in March, phosphorus in August and chloride in January. This is in distinct contrast to the other six sites, where many of these variables increased and decreased together.

5.53 Zinc, cadmium, barium and lead

Seasonal trends for these metals were rarely consistant within sites, nor did any one element exhibit a general trend which was common to all streams. In general, metal accumulation by Rhynchostegium in the four upland streams followed the seasonal variation in aqueous metals most closely in the period spring-early autumn. The seasonal plots of metals in these two components (Figs. 5.04, 5.08, 5.12, 5.16) clearly show that departures from this close relation were most likely to occur in the months from November through February. This was a period of unpredictable changes in flow (due to rain and snow melt) and consequent changes in pH, phosphorus, calcium and so on. Spring, summer and early autumn were in large part periods of more stable physical and chemical conditions. Temporal correlations with monthly accumulation ratios support this as variations in either aqueous sodium, calcium or magnesium (- effect) and organic phosphorus (+ effect) were involved. Potassium (-), manganese (+) and iron (+) in the moss were also indicated as seasonally important factors for some of these populations.

Metal accumulation in the lowland rivers was less consistent. Accumulation by mosses in the River Team upstream of the metal pollution (0024-05; Fig. 5.20) varied mostly according to seasonal events, similar to that observed in upland streams. Below the input of the zinc effluent and more sewage (0024-20; Fig. 5.24), accumulation was governed by the severe decline in water quality, as caused by the closure of the colliery and its dilution effect. Although zinc was the principal metal to have increased (by a factor of 10 - 30 times), cadmium, barium and lead also increased. Thus, seasonal events were largely masked by anthopogenic factors. Zinc accumulation by the two mosses nonetheless closely followed this erratic increase in aqueous concentrations. Aqueous heavy metals in the River Wear, Shincliffe (0008-65; Fig.5.28) were among the lowest of the sites studied and seasonally the least variable. However, concentrations of four heavy metals in mosses increased over autumn - winter and were lowest in the spring and summer. Certain water chemistry variables, such as pH, phosphorus and calcium varied in an inverse pattern to metal accumulation.

5.54 Differences between species

The decline and near absence of Amblystegium from the Team during December and January suggests that this species was "less perennial" than either Rhynchostegium or Fontinalis; its abundance was markedly higher during the summer months. On average Rhynchostegium riparioides accumulated metals (apical tips) in concentrations roughly 20% greater (on a dry weight basis) than Amblystegium riparium and nearly four times more than in Fontinalis antipyretica, in the two rivers studied. Temporal correlations have shown that pairs of species (A.r., R.r. in Team; F.a., R.r. in Wear) responded similarly to seasonal changes in aqueous metals. In general, accumulation by the three species was influenced by similar factors, in particular aqueous magnesium and calcium and total alkalinity.

5.55 Hypotheses

The influence of temporal factors on metal accumulation by aquatic mosses have been shown to be important. Some of this effect was found to be related to seasonal fluctuations in environmental factors which in turn correlated with variations in metal accumulation. The analysis identified chemical characteristics of the mosses, especially potassium, manganese and iron which should be examined further. The fact that enrichment ratios correlate with several seasonally varying factors suggests that they were not constant.

It is hypothesized that the principal factors leading to seasonal differences in metal accumulation by aquatic bryophytes are water chemistry variables, particularly magnesium, calcium and filtrable reactive phosphate. It is also suggested that differences may exist in metal accumulation between different populations of the same species. From the time-series analysis, there was little evidence to suggest seasonal differences in the mosses may influence metal accumulation.

CHAPTER 6. BIOMETRIC ANALYSIS OF MORPHOLOGICAL VARIATION IN RHYNCHOSTEGIUM RIPARIOIDES

6.1 Introduction

Observations made on several populations of Rhynchostegium riparioides during reconaissance surveys indicated a wide range of morphological variation within this species. As mentioned previously (section 2.72), Rhynchostegium may adopt in certain rivers a morphology similar to Amblystegium riparium or to aquatic species of Brachythecium and Hygrohypnum. To examine this variability, 105 different populations of Rhynchostegium were collected for herbarium specimens during the intensive river survey (chapter 4).

The 15 morphological characters measured and analysed have been outlined and explained in Methods (section 2.82). Results of these measurements are presented in section 6.2. Figures are presented which illustrate the extremes of the variation, as well as "typical" morphologies. In the analysis (section 6.3), relationships between certain morphologies and the environmental conditions where they prevail, are considered. Finally, a biometric classification of all populations using a cluster analysis, is presented (section 6.4). The effects these morphological differences may have on metal accumulation are also considered in this section, based on the characters indicated by cluster analysis.

6.2 Morphological characters

6.21 "Typical" plants and variation

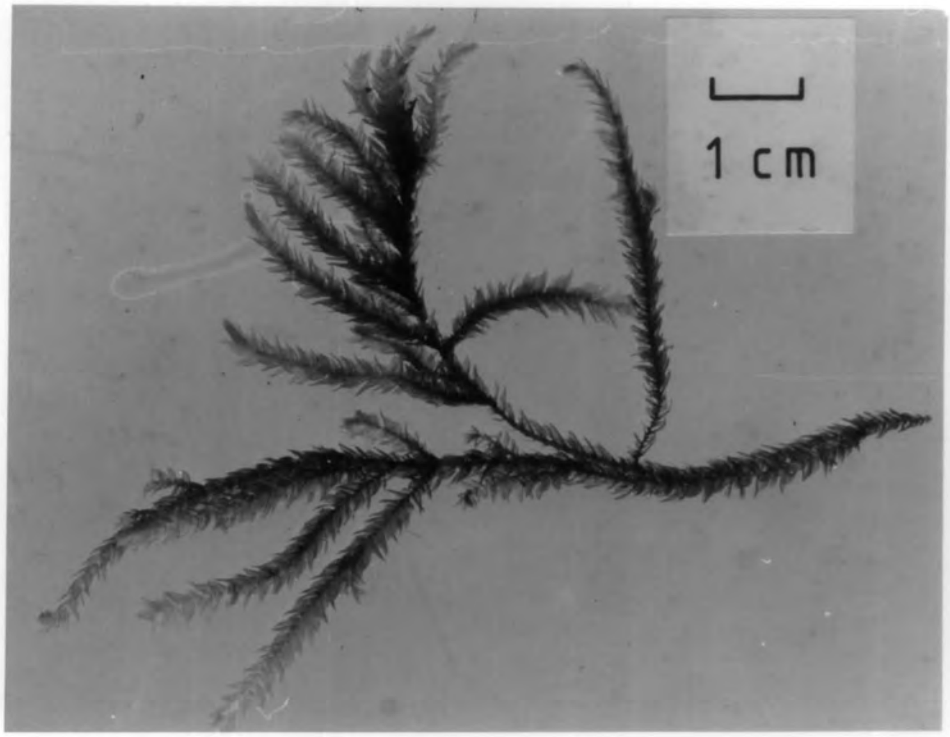
Although many bryophyte floras (e.g. Nyholm et al., 1954-1969; Smith, 1978; Crum & Anderson, 1981) discuss morphological variability in Rhynchostegium riparioides, some definite characters have been recognized. "Typical" plants were usually robust, with a firm or rigid texture. Colour was usually bright or deep green above, often blackish below. Branches frequently were long, although they could often be irregular, from parallel to pinnate, illustrated by the plants in Fig. 6.01. Most authors describe the leaves as broadly ovate, concave and erect, so that they stand out distinctly from the stems. However, Wilson (1855) mentions they frequently may be complanate. Leaf margins were typically regularly denticulate (but unbordered) over the majority of the length (Fig. 6.02). A single or sometimes slightly branched (never double) nerve usually extended just below the apex. Mid-leaf cells were linear; angular cells were rectangular and chlorophyllose. Sporophytes (Fig. 6.03) were diagnostic, having a smooth, red or purplish seta which extends up to 2 cm in length. The ovate capsule was typically covered by a strongly rostrate operculum (lid). Thus, both the English name, "Long-beaked Water Feather Moss," (Wilson, 1855) and variously applied generic names (-rhynch: Greek meaning "beak") refer to this characteristic appearance.

6.22 Descriptive statistics of morphological variability

Of the characters described, several remained constant among the populations sampled during the intensive survey. Sporophytes, when present, were always of the typical form, including seta colour, capsule shape and shape of the lid. Mid-leaf and angular cell shapes

Figure 6.01. Example of a typical plant of <u>Rhynchostegium</u> <u>riparioides</u>.

Figure 6.02. Example of typical leaves of <u>Rhynchostegium</u> <u>riparioides</u>.


1 cm

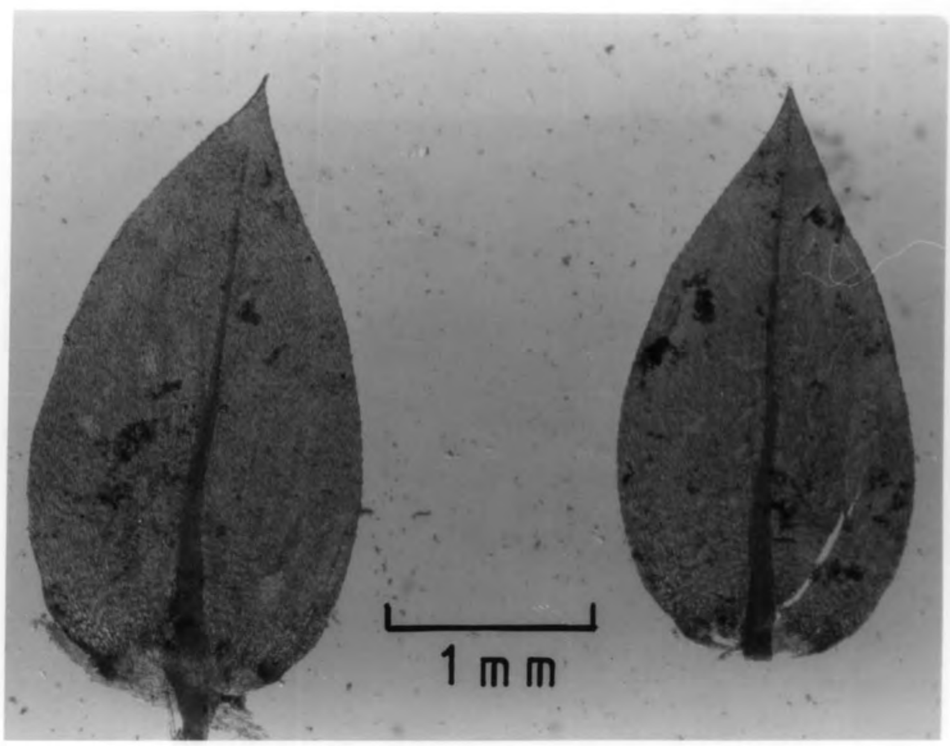
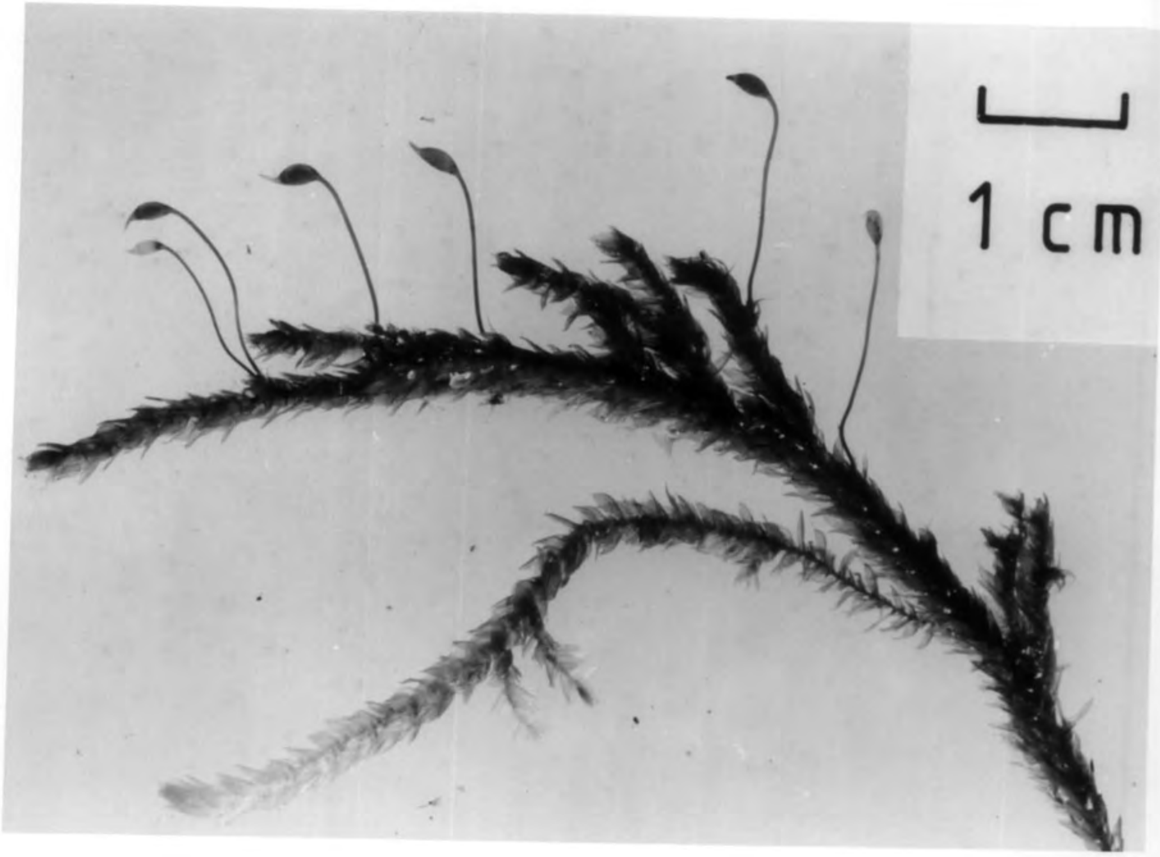
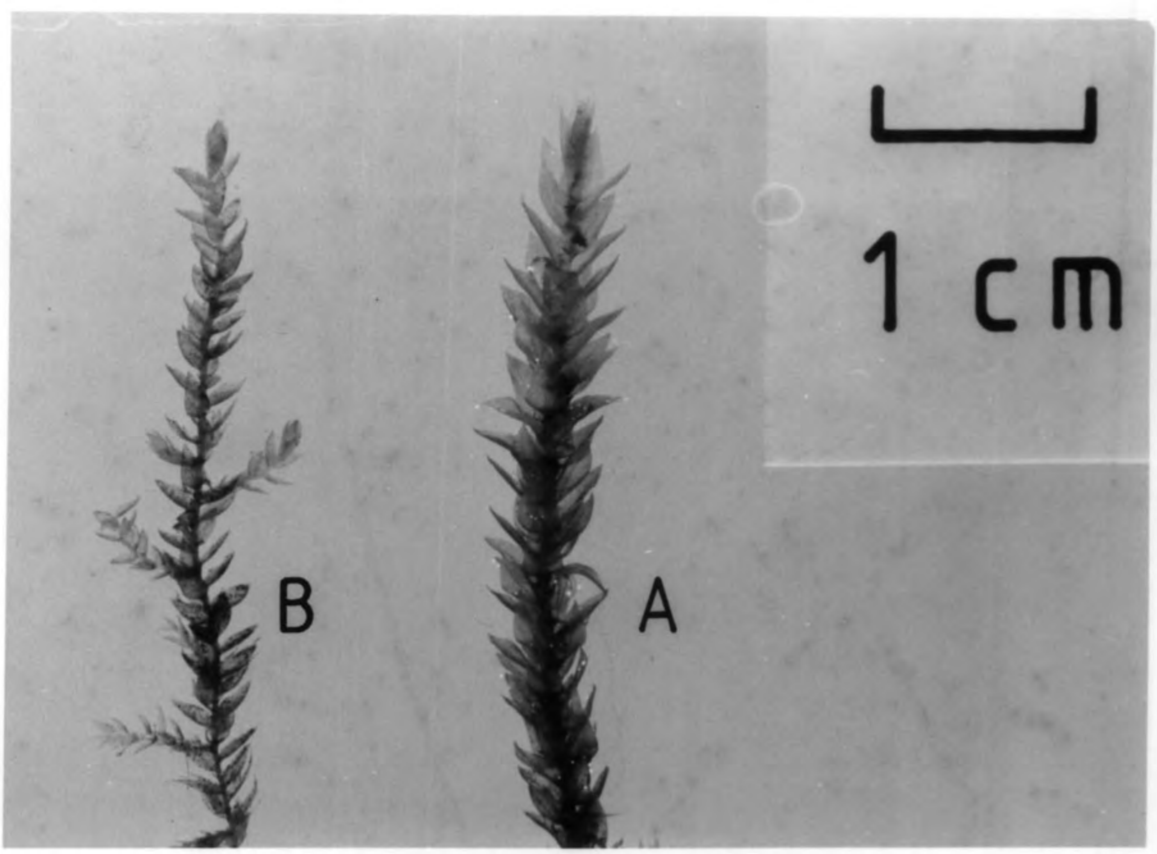

1 mm

Figure 6.03. Sporophytes of Rhynchostegium riparioides.

Figure 6.04. Examples of "robust" (type A) and "flaccid" (type B) Rhynchostegium plants.


1 cm


1 cm
B
A

of the leaves were also unvaried. Variation was commonly expressed in the texture, branching and colour of the plants. Leaves also varied in size and shape, as well as the degree to which they stood out from the stems. Two extremes (Fig. 6.04) were the large, highly robust plants, with infrequent parallel branches (e.g. "Race Fell Burn," 0310-90) and smaller, flaccid plants, with reduced leaves (e.g. River Etherow, 0255-25).

Morphological characters used to score the moss populations included attributes of both plants and leaves. Of these, seven continuous variables were measured (Table 6.01). The maximum length of

Table 6.01. Descriptive statistics for continuous biometric characters of 105 populations of Rhynchostegium.

character	n	min	max	$\bar{x}$	SD
plants					
1. max primary axis length (cm)	105	5.0	22.4	10.3	2.5
2. max leafy axis length (cm)	105	2.5	15.8	6.4	2.2
3. max primary node length (cm)	105	1.5	9.8	4.1	1.4
4. mean apical tip weight (mg)	105	0.55	3.26	1.76	0.56
leaves					
5. mean leaf length (mm)	105	1.8	3.2	2.7	0.3
6. mean leaf width (mm)	105	0.8	1.8	1.3	0.2
7. mean breadth (ratio)	105	0.38	0.61	0.49	0.05

primary axes (main stems) varied considerably. Plants exceeded 15 cm in length in several populations, but in many others were less than 10 cm at maximum. The mean weight of a 2 cm apical tip of Rhynchostegium varied by a factor of five and average dimensions of leaves by a factor of two.

Eight discrete (ranked) characters were also scored (Table 6.02). Notable in these results was that angular cell shape was consistently

Table 6.02. Descriptive statistics for discrete biometric characters of 105 populations of Rhynchostegium. For explanation of ranks see section 2.81.

character	min rank	max rank	number per rank 1	2	3	4	5
plants							
robustness	1	5	8	8	31	36	22
colour	1	3	35	53	17	-	-
leafyness	1	3	15	44	46	-	-
branching	1	3	12	92	1	-	-
leaves							
leaf shape	1	3	15	85	5	-	-
margin denticulation	1	4	5	2	21	77	-
nerve length	1	3	1	7	97	-	-
angular cell shape	1	3	105	0	0	-	-

rectangular in all populations, none being hexagonal (similar to Amblystegium riparium) or inflated (similar to Hygrohypnum spp.). "Robustness," which was a subjective estimate including plant texture and erectness of the leaves, was also quite varied. Roughly half the populations (n = 57) were of intermediate or more flaccid textures, unlike that which is regarded as typical of the species. A total of 17 populations were actually brownish rather than some shade of green. A fourth of all populations (n = 28) possessed leaves which were denticulate along two-thirds or less of their length (ranks 1 - 3), which is also atypical of the species. Because angular cell shape was unvaried among the populations sampled, this character was not included in subsequent analyses. The measurements and ranks of the remaining 14 characters have been used in the statistical analyses following, section 6.3.

6.3 Ecotypic variation

In the course of the intensive survey, it became apparent that certain morphological types were found in certain streams or rivers. The highly robust form with larger leaves and frequently long stems (Fig. 6.04, type A) was most commonly observed in springs, spring-fed streams and upland streams in calcareous districts. The opposite extreme (Fig. 6.04, type B) with smaller leaves and more flaccid texture was commonly collected from larger rivers, particularly those which were organically polluted. While these observations were true in most cases, there were several exceptions. Distinctly non-robust, small-leaved forms were collected from Red Tarn Beck (0292-98) in the Lake District and from the River Derwent, downstream of the reservoir (0061-50).

To examine the effects of environmental conditions on morphological variation, biometric characters were correlated against environmental variables measured for the streams from which they were collected. Several physicochemical variables correlated significantly with a number of biometric measurements (Table 6.03). The factor which correlated most strongly was ammonia. Mean apical tip weight, leaf length, margin denticulation, and nerve length were all significantly ($p < 0.001$) less in rivers with greater aqueous ammonia. Filtrable reactive phosphate was similarly, although less strongly, correlated.

Table 6.03. Correlations between physiochemical variables (normalized: see section 4.23) and 14 biometric characters of 105 populations of Rhynchostegium (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

	O.D. 240	pH	tot alk	NH_4	NO_3	FRP	F	Si	SO_4	Cl
axis len	0.05	0.16	0.17	-0.24*	-0.10	-0.13	0.08	0.01	0.02	-0.02
leafy len	0.01	0.02	0.17	-0.01	0.13	0.08	0.01	0.18	0.18	0.17
node len	0.19*	-0.14	-0.01	0.22*	0.09	0.15	-0.15	0.25**	0.04	0.07
tip wt	-0.07	0.22*	0.24*	-0.58***	-0.40***	-0.51***	0.29**	-0.24*	0.08	-0.30**
robust	0.12	0.17	0.20*	-0.55***	-0.40***	-0.44***	0.21*	-0.18	-0.17	-0.33***
colour	0.23*	0.07	-0.10	-0.21*	-0.31**	-0.20*	-0.01	-0.30**	-0.29**	-0.21*
leafy	0.01	0.01	0.07	0.27**	0.27**	-0.31**	-0.03	0.25*	0.23*	0.31**
branch	-0.08	-0.12	-0.16	0.15	0.29**	0.17	0.02	0.10	0.16	0.22*
leaf len	0.13	0.27**	0.21*	-0.43***	-0.35***	-0.36***	0.27**	-0.26**	-0.02	-0.17
leaf wid	0.10	0.18	0.24*	-0.37**	-0.32***	-0.38***	0.22*	-0.17	0.07	-0.16
breadth	0.01	0.01	0.16	-0.12	-0.14	-0.21*	0.08	0.02	-0.08	-0.10
lf shape	0.03	-0.08	-0.20*	0.30**	0.29**	0.35***	-0.10	0.10	0.09	0.15
marg dent	0.13	0.30**	0.33**	0.33***	-0.66***	-0.50***	-0.44***	0.25**	-0.17	-0.42***
nerv len	0.06	0.10	0.22*	-0.46***	-0.27**	-0.36***	0.21*	-0.11	-0.11	-0.24

None of the physical or chemical variables which were measured correlated significantly with the maximum lengths of primary or leafy axes.

Correlations between these same morphological characters and aqueous metals (Table 6.04) revealed that very few characters correlated significantly with concentrations of aqueous zinc, cadmium, barium or lead. The stongest correlation between biometric characters and any single element was with sodium. Many of these were the same characters which correlated negatively with ammonia. Although previous field observations identified robust plants in many calcareous districts, correlations with aqueous calcium were mostly non-significant. However, negative and positive correlations with the ratio of Na:Ca in streamwater were all greater than for sodium alone.

A single set of correlations were calculated between scores on the subjective plant colour scale and concentrations of metals in apical tips of <u>Rhynchostegium</u> (Table 6.05). It should be noted that

Table 6.05. Correlations between metals in 105 populations of <u>Rhynchostegium</u> and the subjective colour index of plants (colour = pale to bright green, medium to dark green, brownish green to black; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

element	r
Na	-0.36 ***
Mg	-0.27 **
K	-0.43 ***
Ca	0.15
Mn	0.35 ***
Fe	0.10
Zn	0.05
Cd	-0.04
Ba	0.08
Pb	-0.01

Table 6.04. Correlations between aqueous metals (normalized: see section 4.23) and 14 biometric characters of 105 populations of <u>Rhynchostegium</u> (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

	Na	Mg	K	Ca	Mn	Fe	Zn	Cd	Ba	Pb	ratio Na:Ca
axis len	-0.08	0.02	-0.01	0.16	-0.15	-0.10	0.01	-0.06	0.18	0.05	-0.17
leafy len	0.14	0.18	0.10	0.22*	0.04	-0.13	-0.02	-0.09	0.12	-0.02	-0.06
node len	0.04	0.05	0.01	0.03	0.13	0.10	0.02	-0.11	0.07	0.03	0.05
tip wt	-0.32***	-0.09	-0.12	0.15	-0.35***	-0.32***	0.18	0.23*	0.15	0.15	-0.41***
robust	-0.35***	-0.10	-0.22*	0.09	-0.41***	-0.18	-0.01	0.03	0.17	0.21*	-0.43***
colour	-0.20*	-0.28**	-0.16	-0.20*	-0.20*	0.10	-0.19*	-0.12	-0.04	-0.16	-0.14
leafy	0.34***	0.23*	0.22*	0.12	0.24*	0.08	-0.04	-0.16	0.06	-0.12	0.21*
branch	0.25*	0.10	0.21*	-0.07	0.22*	0.03	0.06	0.16	-0.15	-0.04	0.34***
leaf len	-0.19*	0.01	-0.05	0.16	-0.20*	-0.05	0.06	-0.01	0.06	0.04	-0.26**
leaf wid	-0.17	-0.01	-0.10	0.16	-0.26**	-0.16	0.01	-0.02	0.15	0.02	-0.29**
breadth	-0.07	0.01	-0.09	0.08	-0.17	-0.18	-0.01	0.02	0.17	0.06	-0.16
lf shape	0.15	-0.01	0.07	-0.11	0.16	0.22*	-0.14	-0.12	-0.16	-0.15	0.26**
marg dent	0.40*	0.11	0.22*	0.20*	-0.39***	-0.12	0.08	0.18	0.28**	0.30**	-0.56***
nerv len	0.19*	-0.10	-0.12	0.14	-0.25**	-0.12	0.07	0.14	0.11	0.24*	-0.36***

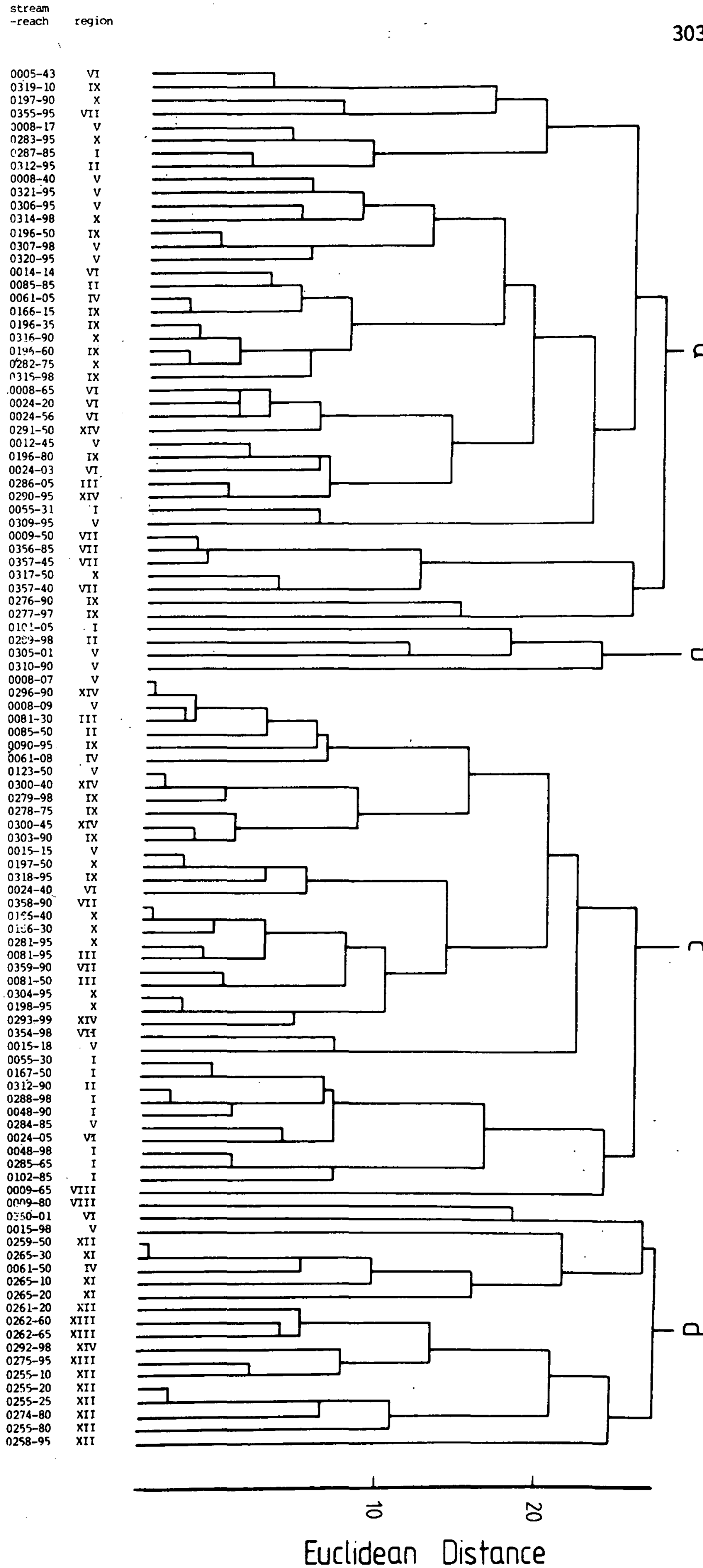
correlations using discrete variables are approximate. The only metal positively (and significantly) correlated with darker colour was manganese in the plant. Hence, the frequently cited "taxonomic" character of dark brown or blackish stems in R. riparioides may have been a function of plant and/or water chemistry.

6.4 Biometric Analysis

A classification of Rhynchostegium populations based on biometric characters, was carried out in order to determine whether distinct groups or "morphotypes" exist. Two of the original 15 characters were excluded. Angular cell shape was left out as it was constant throughout all populations. The subjective colour estimate was removed, in light of results in section 6.3 and experimental results to follow (sections 7.28, 7.333), as it was regarded not to be a character of the plants themselves. Measurements on the remaining 13 characters, in a matrix of 105 cases (populations) were used. The algorithm which produced the most meaningful and distinct clusters was a complete linkage based on standardized measurements (to equalize differences in scale), measured in Euclidean distance.

Cluster analysis (Fig. 6.05) revealed four groups of populations of approximately equal internal similarity. Group "a" consisted of robust plants of average size and tip weight, whose leaves are long and broad. Populations from this group and groups "b" and "c" all possessed well denticulated leaves with strong nerves. Group "b" was a small group of populations with large leaves similar to group "a", but were very long plants with a highly robust texture and greater than average weight for

Figure 6.05. Dendrogram from cluster analysis of 105 populations of Rhynchostegium based on 13 morphometric charcters (each population is indicated by stream & reach no. and hydrogeological region).


stream -reach
region
0005-43 VI
0319-10 IX
0197-90 X
0355-95 VII
0008-17 V
0283-95 X
0287-85 I
0312-95 II
0008-40 V
0321-95 V
0306-95 V
0314-98 X
0196-50 IX
0307-98 V
0320-95 V
0014-14 VI
0085-85 II
0061-05 IV
0166-15 IX
0196-35 IX
0316-90 X
0196-60 IX
0282-75 X
0315-98 IX
0008-65 VI
0024-20 VI
0024-56 VI
0291-50 XIV
0012-45 V
0196-80 IX
0024-03 VI
0286-05 III
0290-95 XIV
0055-31 I
0309-95 V
0009-50 VII
0356-85 VII
0357-45 VII
0317-50 X
0357-40 VII
0276-90 IX
0277-97 IX
0101-05 I
0289-98 II
0305-01 V
0310-90 V
0008-07 V
0296-90 XIV
0008-09 V
0081-30 III
0085-50 II
0090-95 IX
0061-08 IV
0123-50 V
0300-40 XIV
0279-98 IX
0278-75 IX
0300-45 XIV
0303-90 IX
0015-15 V
0197-50 X
0318-95 IX
0024-40 VI
0358-90 VII
0196-40 X
0186-30 X
0281-95 X
0081-95 III
0359-90 VII
0081-50 III
0304-95 X
0198-95 X
0293-99 XIV
0354-98 VII
0015-18 V
0055-30 I
0167-50 I
0312-90 II
0288-98 I
0048-90 I
0284-85 V
0024-05 VI
0048-98 I
0285-65 I
0102-85 I
0009-65 VIII
0009-80 VIII
0350-01 VI
0015-98 V
0259-50 XII
0265-30 XI
0061-50 IV
0265-10 XI
0265-20 XI
0261-20 XII
0262-60 XIII
0262-65 XIII
0292-98 XIV
0275-95 XIII
0255-10 XII
0255-20 XII
0255-25 XII
0274-80 XII
0255-80 XII
0258-95 XII
a
b
c
d
10
20
Euclidean Distance

apical tips. Group "c" had longer, but less broad leaves than in groups "a" and "b". These were plants of average length and weight, and ranged from intermediate to moderately robust in texture. Group "d" were small to average sized plants with a flaccid to intermediate texture. Leaves in these populations tended to be smaller than average, with a short nerve and poor denticulation.

Populations from nearly every hydrogeological region were represented in each of the four clusters, suggesting a lack of any obvious geographical cline. The most restricted cluster, group "d," consists primarily of populations from the Holme (XI), Mersey (XII) and Ribble (XIII) catchments, although single populations from each of the Derwent (IV), Weardale (V), Lower Tees (VIII) and Lake District (XIV) regions were also included. Similar numbers of populations from Weardale, Teesdale, Arkengarthdale and Swaledale are separated into the two main groups, "a" and "b". However, the majority of the lowland Durham populations are within group "a" and the majority of the Alston Moor populations in group "c". No lowland populations from any region were represented in cluster "b", which contains the largest, most robust plants.

As some distinctions could be made between certain forms or ecotypes in R. riparioides, it was of interest to know whether specific morphologies accumulated metals differently. As concentrations of metals in streamwater differ among the streams sampled (section 4.22), correlations were made between enrichment ratios for each population and the key biometric characters suggested by cluster analysis (Table 6.06). Only one statistically significant (although small) correlation

Table 6.06. Correlations between key biometric characters in 105 populations of Rhynchostegium and enrichment ratios for zinc, cadmium, barium and lead in apical tips (* = $p < 0.05$).

	enrichment ratio			
character	Zn	Cd	Ba	Pb
max axis length	-0.15	-0.15	-0.14	-0.14
max leafy length	0.14	-0.10	0.11	-0.05
robustness	0.07	-0.02	0.05	0.08
mean tip weight	-0.01	-0.23*	-0.03	0.11
mean leaf length	0.04	-0.07	-0.12	-0.08
mean leaf width	0.01	-0.05	-0.08	0.01
mean leaf breadth	-0.06	-0.14	-0.03	0.08
margin denticulation	0.01	-0.16	-0.02	0.07
nerve length	0.06	-0.04	0.03	0.05

was found: a negative relation between greater tip weight and cadmium accumulation. Results do not indicate any obvious relationship between morphology and accumulation of heavy metals.

CHAPTER 7. EXPERIMENTAL STUDIES ON METAL ACCUMULATION BY AQUATIC BRYOPHYTES

7.1 Introduction

Experimental studies on metal accumulation followed two lines, one in the field (section 7.2), the other in the laboratory (section 7.3). The majority of the former of field populations into streams of known water chemistry and metal concentrations to examine rates of uptake in nature. Specific manipulations within a single stream were also carried out to compare interpopulation differences and the effects of microhabitat. A few studies using unmodified field populations examined variability in metal concentrations of mosses *in situ* and the localization of certain elements. Laboratory experiments tested the effects of some of the factors suggested by earlier field surveys and field experiments.

Most experiments were carried out using *Rhynchostegium riparioides*. A few studies on variability and uptake were made with other species. ~~species~~ In order to limit the number of experiments and subsequent analyses, interest was focussed on the metal zinc. Field surveys have shown that this element occurred over a fairly wide concentration range (chapters 4, 5) and several factors were suggested to be involved in its uptake. Use of this element had the advantage of being readily available as a radioisotope which has a fairly long half-life (244 days: Wang & Willis, 1965) and may occur as a pollutant in rivers downstream of nuclear reactors (e.g. Davis *et al.*, 1958).

7.2 Field Studies

7.21 Cross-transplants

An experiment was conducted in the River Team between two sites above (0024-05) and below (0024-20) a zinc effluent from a factory which manufactures batteries. Boulders with Rhynchostegium were taken from each site and moved to the other. The sampling routine included an intensive, short-term sampling period (48 h) and a longer period (32 days). Temperature, pH and concentrations of aqueous zinc in the two sites are given in Table 7.01. Although fluctuations in zinc were found

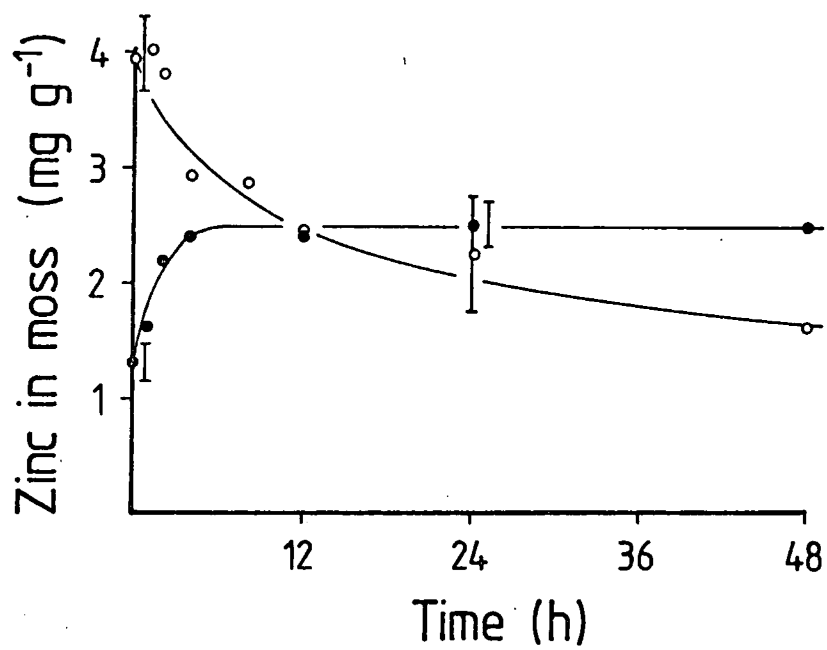
Table 7.01. Temperature (^{o}C), pH and zinc (mg l^{-1}) in stream water at the two transplant sites in the River Team, during the short-term study (* = sampled during spate).

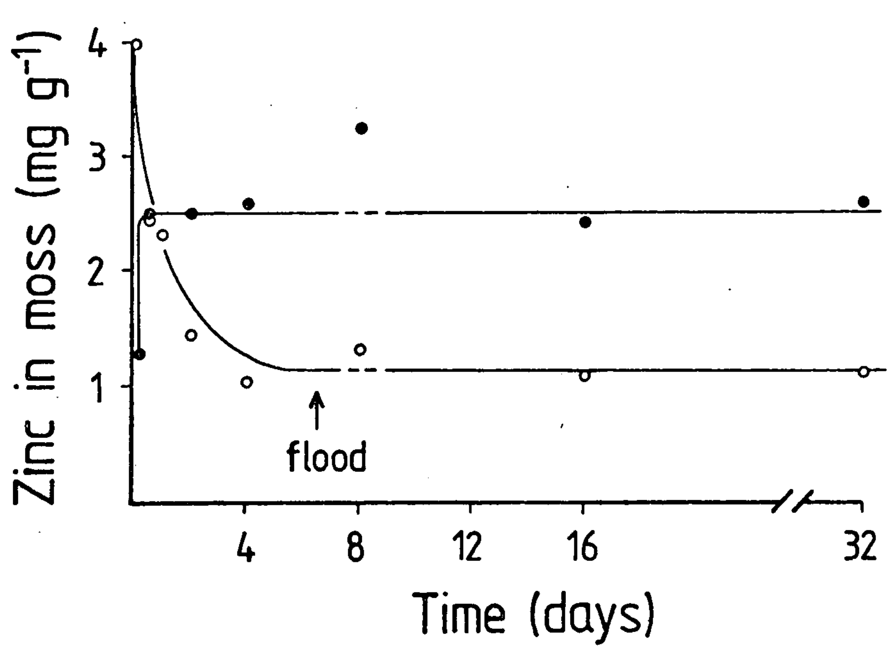
time (h)	Kyo temp	Kyo pH	Kyo Zn(T)	Kyo Zn(F)	Causey temp	Causey pH	Causey Zn(T)	Causey Zn(F)
0	11.8	7.2	0.074	0.056	14.5	7.6	0.38	0.37
0.5	12.0	7.3	0.063	0.049	14.3	7.6	0.37	0.31
1	12.2	7.3	0.049	0.049	14.5	7.5	0.40	0.36
2	12.4	7.4	0.056	0.049	14.5	7.6	0.72	0.66
4*	14.8	7.0	0.186	0.084	14.8	7.7	1.40	1.16
8	13.0	7.1	0.077	0.056	13.5	7.4	0.58	0.38
12	11.4	7.4	0.074	0.063	12.8	7.7	0.74	0.63
24	11.7	7.1	0.088	0.081	12.5	7.6	0.51	0.44
48	13.5	7.5	0.077	0.049	14.5	7.4	0.29	0.23

at both sites, aqueous zinc in the lower reach remained roughly eight times greater than in the upstream site. Results for zinc accumulation (Fig. 7.01) indicate that uptake was extremely rapid, reaching saturation within about 2 - 4 h. In contrast, loss of zinc proceeded less rapidly and had apparently not levelled out after 48 h.

Figure 7.01. Uptake and loss of Zn by Rhynchostegium over short term in cross-transplant experiment.

Figure 7.02. Uptake and loss of Zn by Rhynchostegium over long term in cross-transplant experiment.


Zinc in moss (mg g⁻¹)
4
3
2
1
12
24
36
48
Time (h)


Zinc in moss (mg g⁻¹)
4
3
2
1
flood
4
8
12
16
32
Time (days)

Over the long term, aqueous zinc concentrations tended to drop at both sites (Table 7.02), about 40% at Kyo and 50% at Causey. However,

Table 7.02. Zinc concentrations (mg l^{-1}) in stream water in the two transplant sites in the River Team during the long-term study.

time (days)	Kyo Zn(T)	Kyo Zn(F)	Causey Zn(T)	Causey Zn(F)
0	0.074	0.056	0.38	0.37
1	0.088	0.081	0.51	0.44
2	0.077	0.049	0.29	0.23
4	0.067	0.056	0.34	0.29
8	0.053	0.042	0.25	0.21
16	0.046	0.035	0.26	0.20
32	0.040	0.034	0.21	0.18

the plot of zinc loss and uptake (Fig. 7.02) was more stable, with loss eventually levelling out at about 4 days. A flood during days 6 - 7 (not sampled), although indicated by increases of zinc in both mosses two days later, was not evident from water samples. While mosses from Causey moved to Kyo ultimately reached similar zinc concentrations to the in situ populations (c. 1 mg g^{-1}), net accumulation by the Kyo population ($\bar{x}$ = 2.5 mg g^{-1} Zn) in the contaminated site never reached the concentration measured in the mosses in situ ($\bar{x}$ = 3.9 mg g^{-1} Zn).

7.22 "Nutrient" effects

A pair of transplants were conducted using a population of Rhynchostegium from a reach on the River Browney (0014-14; see Appendices 1 - 3), with low zinc concentrations (T = 0.012, F = 0.009 mg l^{-1} during experiment). One set of boulders plus moss were put into the River Team, Causey (0024-20), designated here as a plus zinc stream

Table 7.03. Temperature (^{o}C), pH and aqueous FRP ($\mu g\ l^{-1}$), calcium and zinc ($mg\ l^{-1}$) in Rookhope Burn (0012-45) and the River Team (0024-20) during the transplant period (blanks = no data available).

time (days)	temp	pH	$[PO_4-P]$	[Ca] T	[Ca] F	[Zn] T	[Zn] F
Rookhope Burn							
0	13.0	7.6	2.0	30.5	30.4	0.27	0.23
1	10.8	7.7		30.6	30.0	0.35	0.31
2				33.0	31.5	0.28	0.20
4	11.4	7.7		35.0	35.1	0.33	0.22
8	11.5	7.7		32.8	32.6	0.29	0.26
16	14.6	7.7		46.6	46.6	0.31	0.22
18	13.8	7.8		35.3	35.4	0.29	0.23
35	13.0	7.8		35.8	34.5	0.42	0.37
64	13.7	7.1		26.3	24.5	0.46	0.42
River Team							
0	15.0	7.4	1410	68.6	68.0	0.28	0.26
1	14.0	7.7		108.0	106.0	0.30	0.25
2				92.6	77.6	0.28	0.26
4	14.3	7.5		108.0	107.0	0.37	0.32
8	14.0	7.3		95.1	92.7	0.25	0.19
16	15.7	7.1				0.55	0.20
18	15.0	7.7		109.0	104.0	0.24	0.22
35	15.0	7.4		67.5	66.8	0.21	0.18
64	14.6	7.7		104.0	102.2	0.29	0.26

with high "nutrient" concentrations (PO_4-P, NO_3-N; Appendix 1: p. 430). The second set were placed in Rookhope Burn (0012-45), an upland stream with lower "nutrients" (Appendix 1). The streams had similar aqueous zinc concentrations (Table 7.03). Mean concentrations in the two sites during the experiment were insignificantly different ($p > 0.05$). However, it was found that aqueous calcium concentrations were significantly greater ($p < 0.01$) in the Team, in addition to phosphate and nitrate. Zinc accumulation by Rhynchostegium in the two streams differed considerably (Fig. 7.03). Mosses transplanted into Rookhope Burn accumulated approximately 35% more zinc than in the River Team. Unlike the previous uptake experiment, this population of Rhynchostegium accumulated similar levels of zinc to what was found in both populations in situ (Table 7.04).

Table 7.04. Comparison of zinc accumulated by Rhynchostegium (mg g^{-1}) in transplanted and in situ mosses at Rookhope Burn and the River Team (* = no replicates taken).

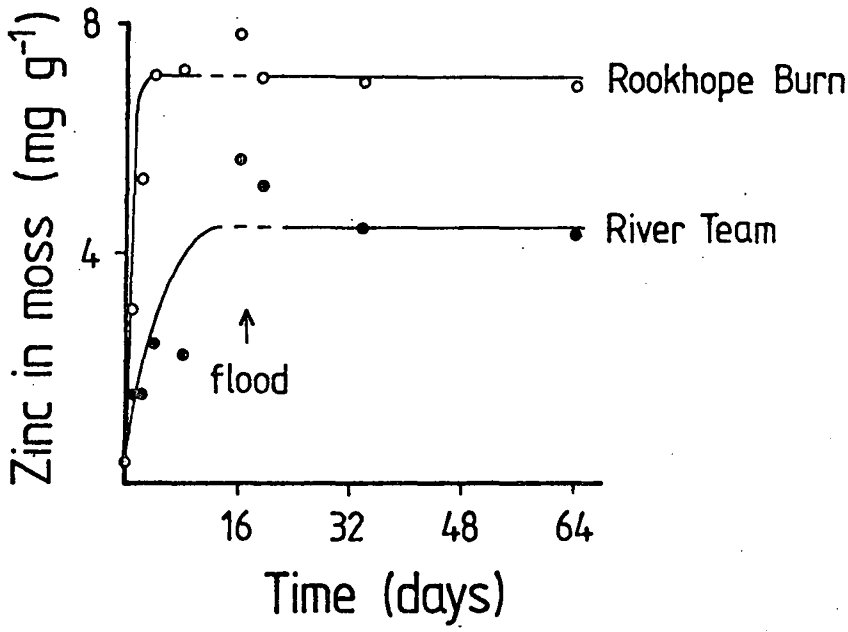
	Rookhope		Team	
	$\bar{x}$	SD	$\bar{x}$	SD
in situ	6.8	*	4.1	0.27
transplant	7.0	0.16	4.4	0.14

7.23 Interpopulation experiments - I

A comparison of metal uptake by different Rhynchostegium populations in the same regime was first made using mosses from five streams each with low aqueous zinc concentrations (Table 7.05). Boulders plus mosses were transplanted into another low metal "control" stream, Waskerley Beck (0123-50; see Appendices 1 - 3). They remained

Figure 7.03. Uptake of Zn by _Rhynchostegium_ transplanted into Rookhope Burn and the River Team.

Figure 7.04. Uptake of Zn by five different _Rhynchostegium_ populations after being transplanted into the River Nent.


Zinc in moss (mg g^{-1})
8
4
Rookhope Burn
River Team
flood
16
32
48
64
Time (days)

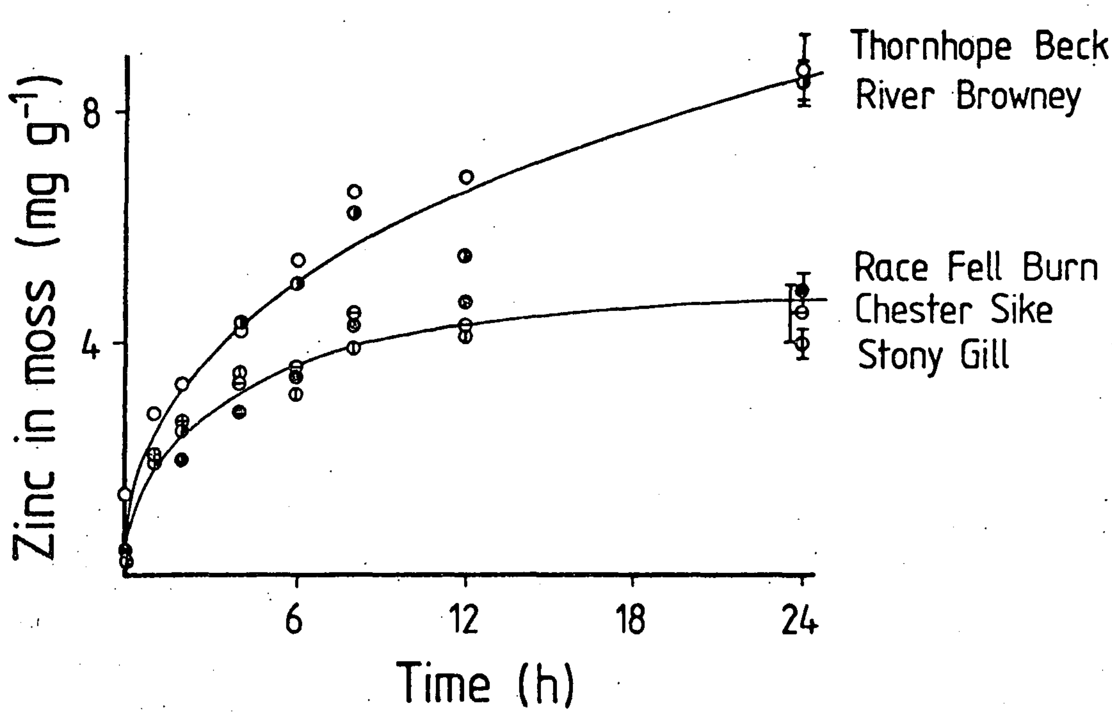

Zinc in moss (mg g^{-1})
8
4
Thornhope Beck
River Browney
Race Fell Burn
Chester Sike
Stony Gill
6
12
18
24
Time (h)

Table 7.05. Sites used for collecting different "low" zinc populations of Rhynchostegium for the first interpopulation study, with pH levels and concentrations of FRP ($\mu g\ l^{-1}$), calcium and zinc (F: $mg\ l^{-1}$) in stream water (* = not sampled).

name	stream -reach	pH	FRP	Ca	Zn survey prelim.	Zn survey final
River Browney	0014-14	8.0	3.3	26	0.009	0.016
Thornhope Beck	0284-85	7.4	< 1.5	50	0.010	0.006
"Race Fell Burn"	0310-90	7.7	< 1.5	18	0.020	0.010
Chester Sike	0355-95	7.8	2.4	30	*	0.026
Stony Gill	0356-85	8.3	2.9	50	0.018	0.010

in this stream for another ten days in order to equalize any differences in metal content prior to the uptake period.

All boulders were then transported into the River Nent (0048-90), which had elevated levels of zinc and other metals from surrounding disused lead and zinc mines. Temperature, pH, and concentrations of aqueous zinc during the experiment are given in Table 7.06.

Table 7.06. Temperature (°C), pH and aqueous zinc concentrations ($mg\ l^{-1}$) in the River Nent (0048-90), during the interpopulation and current velocity experiments.

time (h)	temp	pH	Zn T	Zn F
0	2.4	7.6	1.63	1.57
1	2.5	7.3	1.60	1.53
2	2.7	7.4	1.56	1.53
4	3.5	7.8	1.53	1.50
6	4.5	7.7	1.50	1.45
8	3.5	7.7	1.52	1.50
12	3.8	7.6	1.59	1.55
24	2.5	7.4	1.52	1.48

Concentrations of zinc in stream water were relatively stable and were roughly 100 times greater than concentrations from which the mosses were isolated. Two populations, from Thornhope Beck and the River Browney, accumulated nearly twice the amount of zinc than did the other three populations (Fig. 7.04). Although some variability in uptake was measured, the two groups were significantly different ($p < 0.05$).

7.24 Interpopulation experiments - II

Metal accumulation was also compared in two populations with strongly different experience or "tolerance" to heavy metals (Table 7.07). Tolerance was assumed as zinc-tolerant algal strains had been

Table 7.07. Concentrations (mg l^{-1}) of filtrable zinc and lead in streams used for collecting "tolerant" and "non-tolerant" populations of Rhynchostegium (data from Appendix 3: pp. 427-429).

name	stream -reach	Zn(F)	Pb(F)
"High Crag Burn"	0101-05	0.70	0.014
"Race Fell Burn"	0310-90	0.016	0.002

isolated from the metal contaminated stream (see section 2.414). Boulders with mosses were collected first from the metal contaminated site ("High Crag Burn," 0101-05), transported to the low metal stream ("Race Fell Burn," 0310-90) and remained there for a period of 21 days in order to equalize concentrations of metals in the two populations.

Both populations were then transplanted into the metal contaminated stream and sampled over 24 h. Measurements of pH and aqueous zinc and lead during the experiment are given in Table 7.08. Zinc accumulation

Table 7.08. Temperature (^{o}C), pH, aqueous zinc and lead concentrations (mg l^{-1}) in "High Crag Burn" during the tolerance effects experiment.

time (h)	temp	pH	Zn T	Zn F	Pb T	Pb F
0	11.0	7.5	0.48	0.43	0.012	0.005
1	10.6	7.6	0.48	0.44	0.011	0.006
2	11.0	7.8	0.48	0.44	0.011	0.005
3	11.2	8.0	0.47	0.44	0.010	0.005
4	11.2	8.0	0.47	0.45	0.016	0.005
6	11.1	8.2	0.50	0.44	0.027	0.005
8	11.0	7.8	0.50	0.50	0.010	0.005
10	11.0	7.7	0.50	0.48	0.020	0.005
12	10.5	7.5	0.53	0.48	0.024	0.005
24	10.7	7.7	0.60	0.57	0.011	0.006

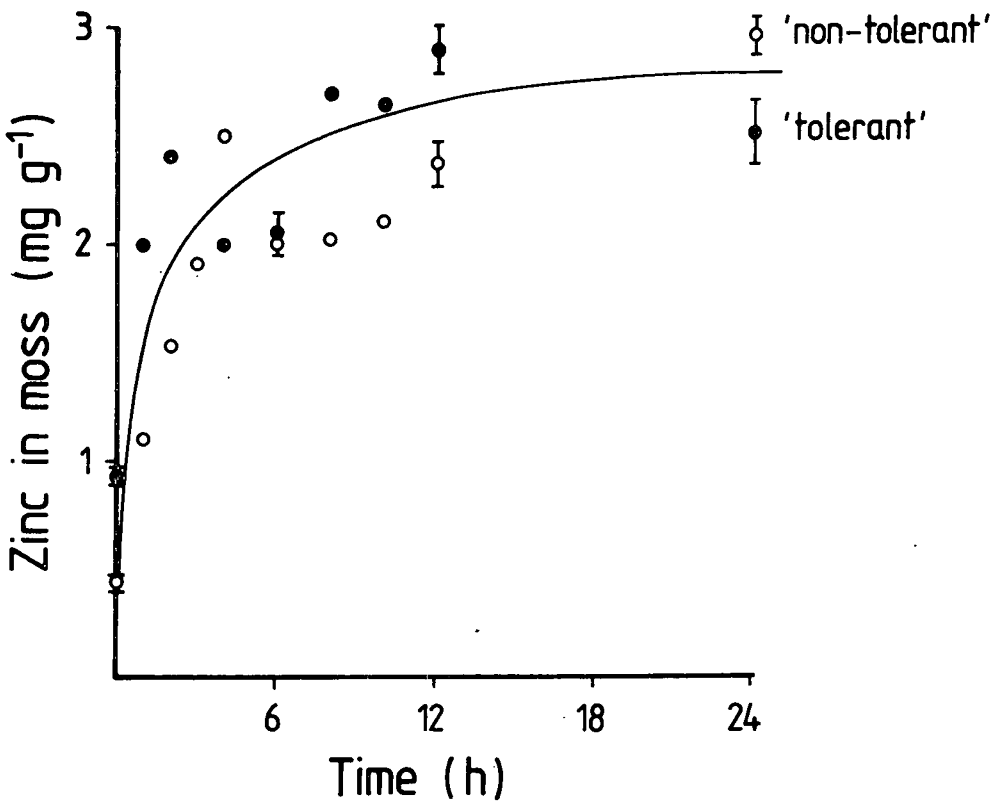
by these two populations were quite similar (Fig. 7.05), although somewhat variable. At 6 h, accumulated zinc in the two were nearly equal, at 12 h roughly 15% less was measured in the "non-tolerant" population, and at 24 h 15% more was measured. A pairwise t-test for all intervals between 6 - 24 h indicate there was no significant difference in accumulation between the two ($p > 0.05$). In contrast, more than twice the lead was accumulated by the "tolerant" population than the "non-tolerant" one over 24 h (Fig. 7.06). This difference was highly significant ($p < 0.01$) and the initial (0 - 4 h) uptake rate for the "tolerant" moss ($y = 0.32x + 0.27$) was roughly five times greater than for the "non-tolerant" moss ($y = 0.057x + 0.16$).

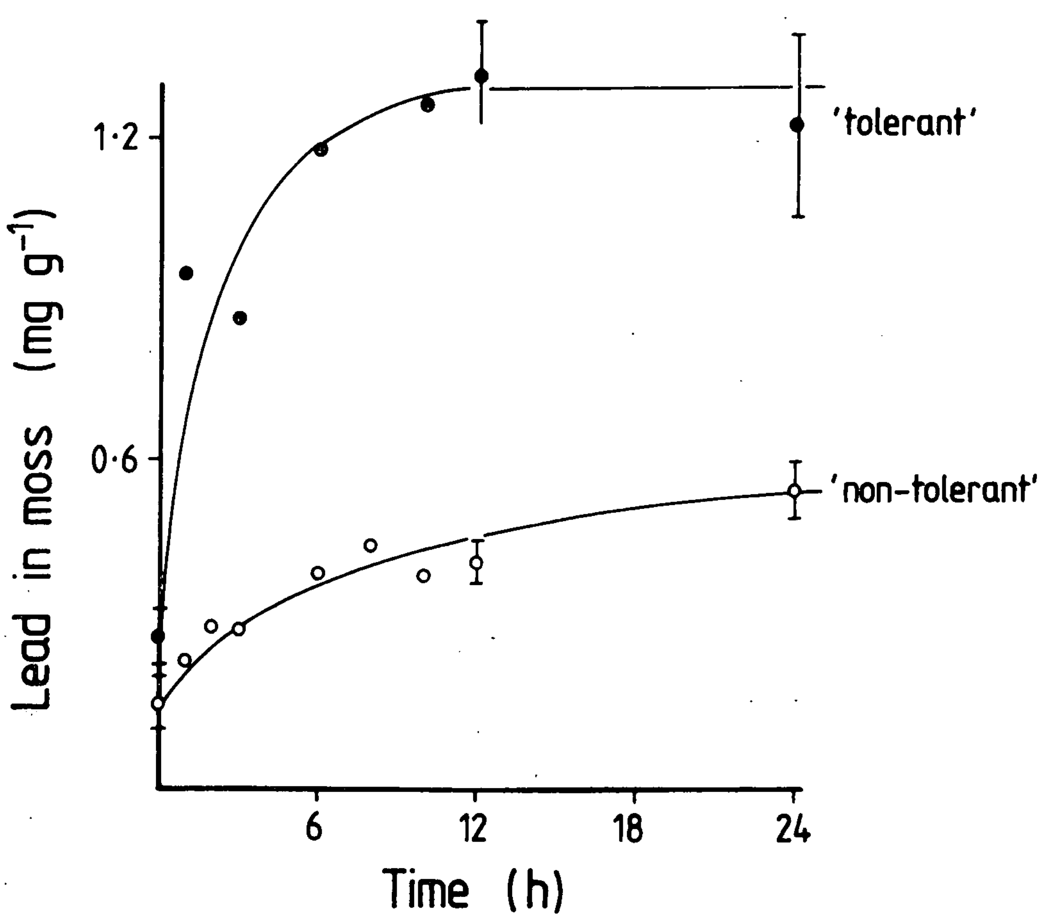
7.25 Current velocity

An experiment testing the effect of current velocity on zinc accumulation was run at the same site (River Nent, 0048-90) where the first interpopulation study was carried out. Boulders with mosses from

Figure 7.05. Uptake of Zn by "metal-tolerant" and "non-tolerant" populations of Rhynchostegium transplanted into "High Crag Burn."

Figure 7.06. Uptake of Pb by "metal-tolerant" and "non-tolerant" populations of Rhynchostegium transplanted into "High Crag Burn."


3
2
1
Zinc in moss (mg g⁻¹)
6
12
18
24
Time (h)
'non-tolerant'
'tolerant'


1·2
0·6
Lead in moss (mg g⁻¹)
6
12
18
24
Time (h)
'tolerant'
'non-tolerant'

"Race Fell Burn" (0310-90) were placed in a pool, negligible current velocity (< 10 cm s^{-1}) and in a riffle ($\bar{x} = 145 \pm 40$ cm s^{-1}). No significant difference in zinc uptake by Rhynchostegium was found (Fig. 7.07) in these two microhabitats over 24 h ($p > 0.05$).

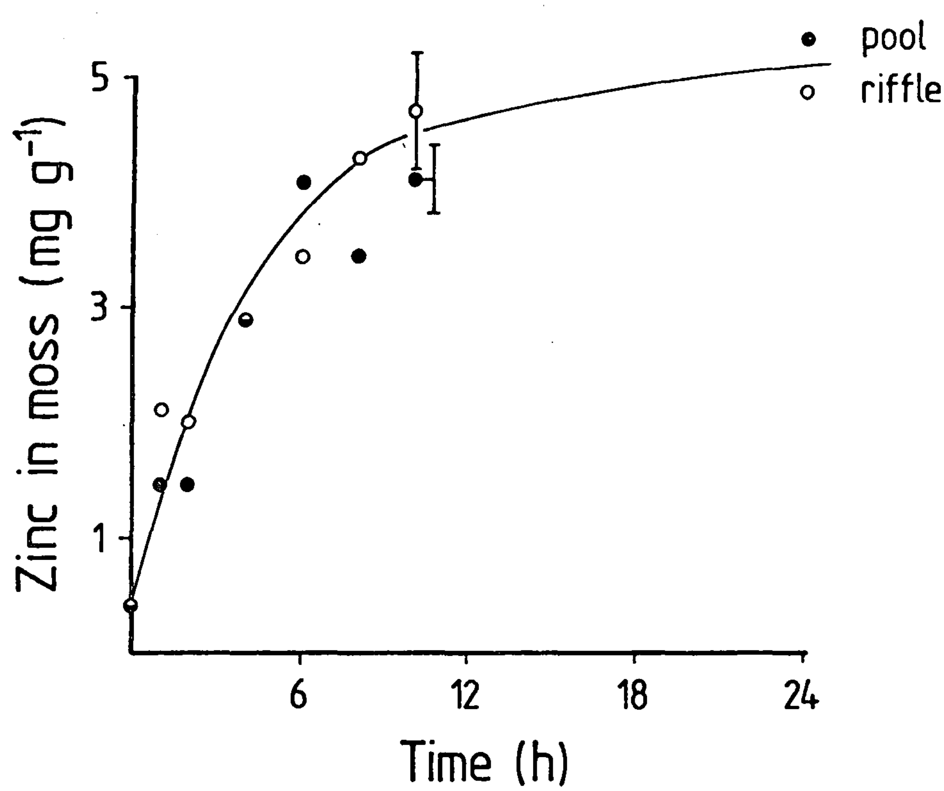
7.26 Variation *in situ*

Samples of mosses from seven different streams (see chapter 5) were collected, with five replicates each from separate boulders within a reach. *Rhynchostegium riparioides* (all sites), *Amblystegium riparium* (one site) and *Fontinalis antipyretica* (one site) were collected. Apical tips (2 cm) were removed and prepared for analysis as described previously (section 2.34). Nine metals were analysed in digests of these mosses and coefficients of variation in these results compared (Table 7.09). Variation was consistently lowest for the metals magnesium, potassium and calcium, while variation in manganese and lead

Table 7.09. Coefficients of variation (%) in metal concentrations from five replicates of 2 cm apical tips of moss from seven stream sites (collections: August 1981 * = data not available).

species	site	Mg	K	Ca	Mn	Fe	Zn	Cd	Ba	Pb
Rhynchostegium riparioides	0101-05	1.4	6.1	2.2	10.2	7.0	3.5	4.7	15.4	11.4
	0289-98	4.4	4.1	3.9	13.4	11.4	3.8	11.0	18.4	9.4
	0085-50	2.9	*	4.2	21.8	21.8	8.5	12.1	12.6	20.8
	0310-90	3.3	5.5	2.8	10.0	27.4	6.7	12.0	5.2	34.5
	0024-05	4.7	8.2	5.3	44.2	4.2	12.4	23.3	17.8	26.9
	0024-20	4.0	4.5	3.3	56.0	4.0	16.5	5.9	28.7	11.2
	0008-65	2.4	4.5	7.5	15.3	21.9	5.5	19.5	11.6	21.0
Amblystegium riparium	0024-20	3.3	6.4	2.0	9.0	3.7	6.3	39.0	37.9	33.3
Fontinalis antipyretica	0008-65	4.5	7.5	4.0	8.8	11.2	10.2	27.9	11.6	13.2

Figure 7.07. Uptake of Zn by Rhynchostegium transplanted into a pool and a riffle in the River Nent.


pool
riffle
Zinc in moss (mg g^{-1})
5
3
1
6
12
18
24
Time (h)

often exceeded 10%. This variation was not necessarily related to absolute amounts of the metal concerned. For example, concentrations of zinc (1.04 mg g^{-1}) and lead (0.124 mg g^{-1}) in Rhynchostegium at Lee Springs (0289-98) were much lower than this moss in "High Crag Burn" (Zn, 7.71; Pb, 3.37 mg g^{-1}), but variation in metal concentrations were quite similar. Variation in most metals in Rhynchostegium and Amblystegium from the same site were similar, although manganese was considerably more variable in the former, and cadmium variability was greater in the latter. Variation between replicates of Fontinalis from the Wear was usually the same or less than in Rhynchostegium, with the exception of cadmium (28% v. 5.5%) which was much higher. In general, zinc was the least variable heavy metal in all three species.

7.27 Localization: stems v. leaves

A comparison was made between concentrations of metals in stems and leaves of Rhynchostegium from "High Crag Burn." Five replicates, each consisting of pooled samples of 20 (stripped) stems or their detached leaves were used. Results (Table 7.10) show that except for potassium,

Table 7.10. Concentrations of selected metals (μg g^{-1}) in detached stems and leaves of Rhynchostegium riparioides from "High Crag Burn" (0101-05).

	stems		leaves	
	$\bar{x}$	SD	$\bar{x}$	SD
K	6300	740	3850	360
Ca	16000	10	14300	1700
Mn	65	20	347	86
Fe	1570	200	9200	1720
Zn	3270	420	6540	640
Pb	264	108	1510	320

concentrations of metals (on a dry weight basis) were greater in the leaves than in the stems. On an absolute basis (content rather than concentration), the difference was greater, as roughly two thirds of the dry weight of a tip consisted of leaves. Leaves contained twice the zinc, and more than five times the lead, by concentration, than stems. T-tests indicated all differences measured, including potassium, were significant ($p < 0.05$).

7.28 Localization: differences along the stem

A second series of experiments was conducted using populations of Rhynchostegium from an uncontaminated site (Lee Springs: 0289-98) and one which was zinc and lead contaminated ("High Crag Burn": 0101-05). Plants were collected and fractionated into successive 1 cm sections down the stem, including the blackened, lower, wirey stems with few or no leaves. Five replicates (of ten sections each) were collected for the first four fractions. Results for the Lee Springs population (Fig. 7.08) indicated marked changes down the stem, with differences between some elements. Patterns of manganese and lead concentrations were similar and increased markedly in the lowest portions. As much as 1.4 mg g^{-1} lead was measured in the lowest cm fraction. Iron increased linearly down the stem, while the trend for zinc appeared to be intermediate between these two patterns. Potassium and calcium increased less dramatically.

Results for Rhynchostegium from "High Crag Burn" (Fig. 7.09) were similar to Lee Springs, although a very close correspondence was found between the patterns for iron and zinc. A peak in these metals occurred in the 5 cm fraction, with a slight decrease further down the stem. The zinc concentration of roughly 6 mg g^{-1} for a "standard" 2 cm apical tip

Figure 7.08. Changes in concentrations of metals in successive 1 cm fractions of Rhynchostegium stems from a low heavy metal stream, Lee Springs.

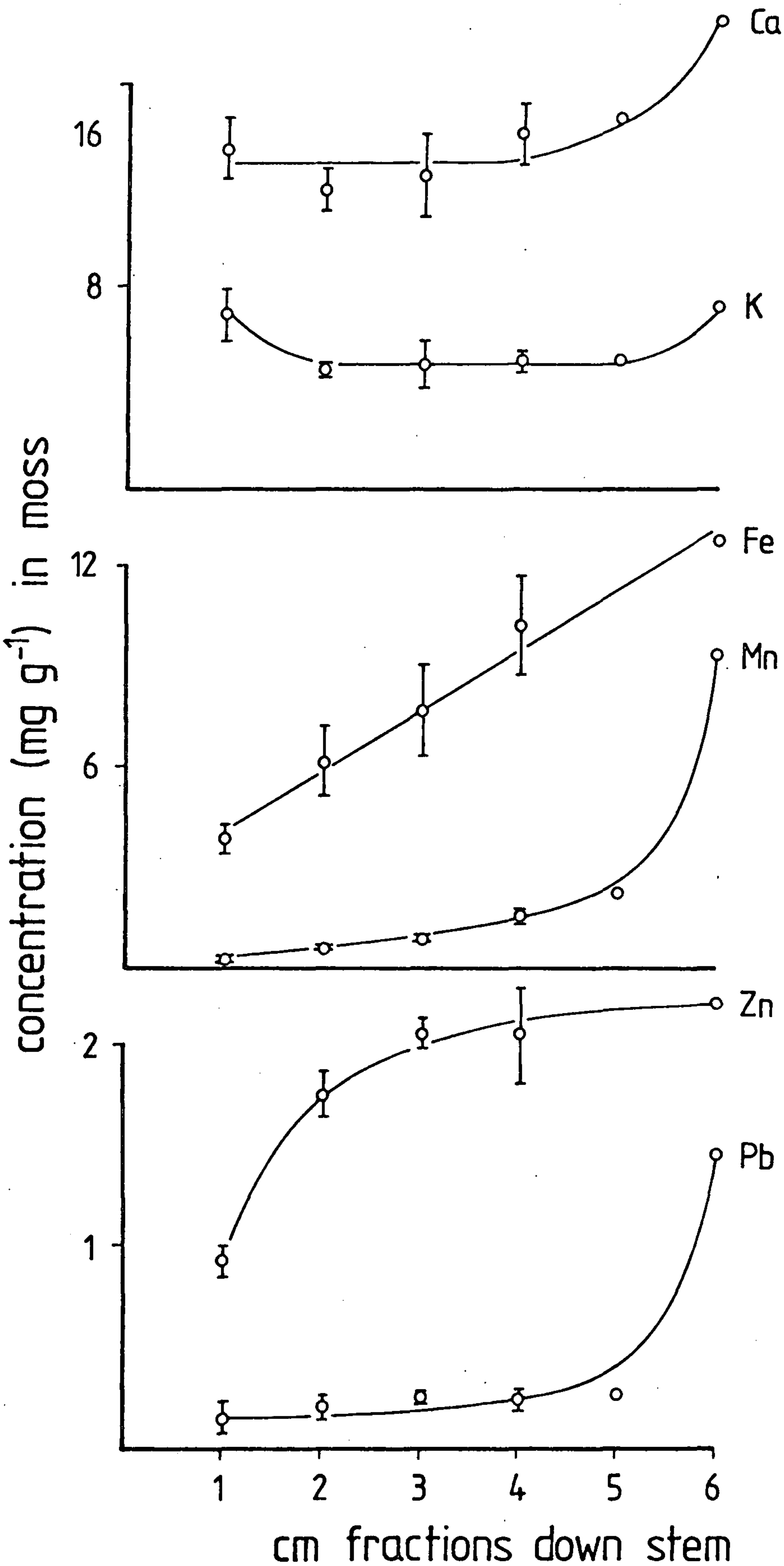
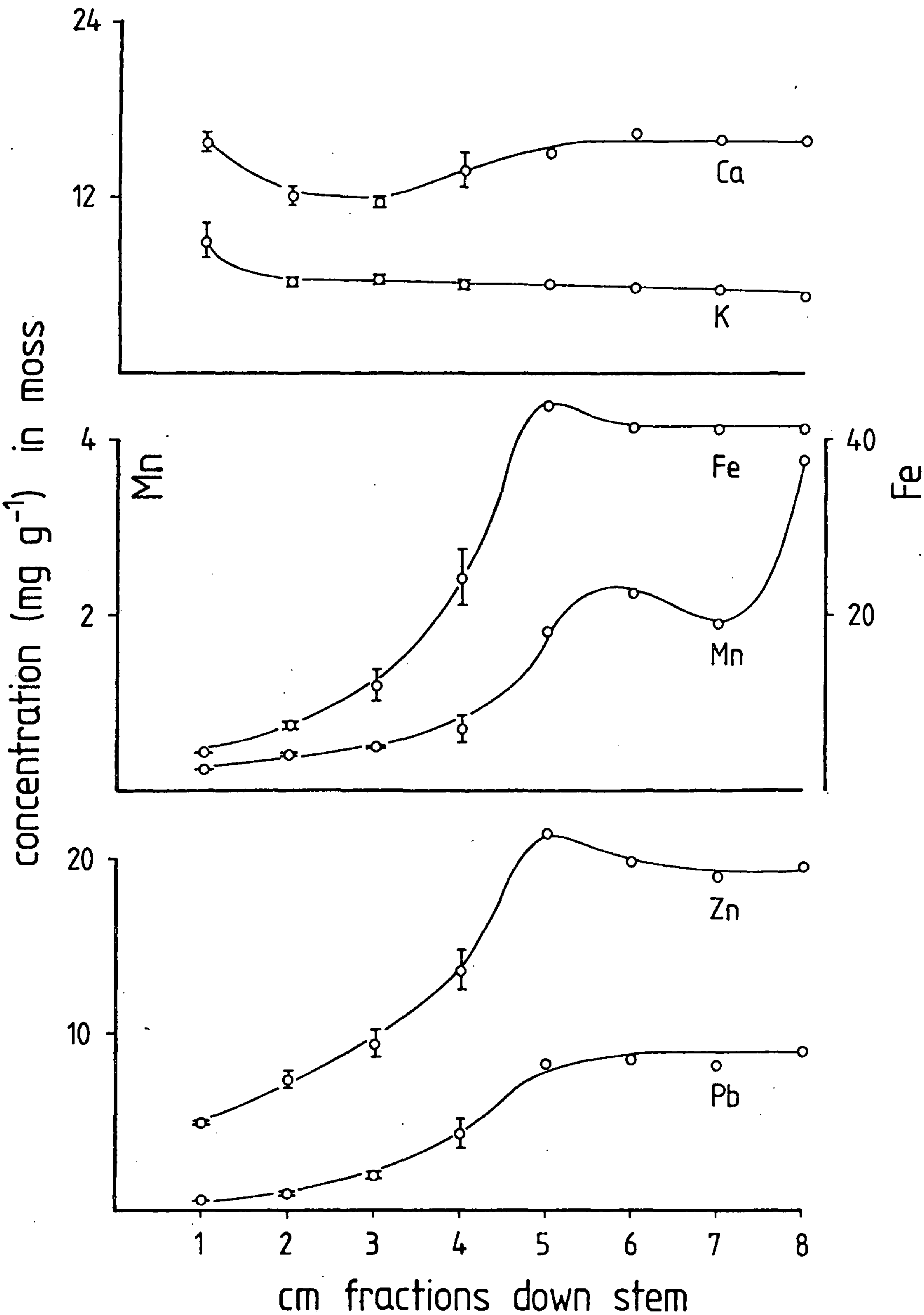

Ca
K
Fe
Mn
Zn
Pb
16
8
12
6
2
1
concentration (mg g-1) in moss
1
2
3
4
5
6
cm fractions down stem

Figure 7.09. Changes in concentrations of metals in successive 1 cm fractions of Rhynchostegium stems from a heavy metal contaminated stream, "High Crag Burn."



24
12
Ca
K
4
2
Mn
Fe
40
20
20
10
Zn
Pb
1
2
3
4
5
6
7
8
concentration (mg g^{-1}) in moss
cm fractions down stem

was less than a third the maximum of 21.5 mg g^{-1} measured in the lower fraction. Manganese and lead also increased along the stem, although their patterns were less similar. Potassium concentrations actually decreased down the stem.

7.3 Laboratory studies

7.31 Preliminary experiments

7.311 Initial uptake rates in the laboratory

These experiments were conducted using non-labelled zinc (i.e. not ^{65}Zn) in the medium. Uptake was measured using 2 cm apical tips of Rhynchostegium, unless stated otherwise. In the first experiment two zinc regimes were used; 0.4 mg l^{-1} and 1.0 mg l^{-1}. Changes in zinc in these media over the 48 h uptake period are given in Table 7.11. No significant change was measured in the regime with 0.4 mg l^{-1}

Table 7.11. Filtrable zinc concentrations (mg l^{-1}) in media during initial accumulation experiment over 48 h (* = not available; n = 5).

time (h)	zinc added 0.40	zinc added 1.00
0	0.40	1.05
1	0.40	1.04
2	0.36	1.04
4	0.40	1.03 ± 0.01
6	*	1.00
8	0.36	1.00
12	0.39	0.95 ± 0.02
24	0.37 ± 0.03	0.86
48	0.42	0.81

zinc and about 20% of the zinc was removed through accumulation by the moss in the 1.0 mg l^{-1} regime. Direct measurement of zinc uptake (Fig. 7.10) indicted that no net accumulation was measured in the more dilute medium. In 1.0 mg l^{-1} zinc, tips reached a concentration of 1.25 mg g^{-1} after 48 h. These concentrations were considerably less than found in transplant experiments with similar concentrations in stream water.

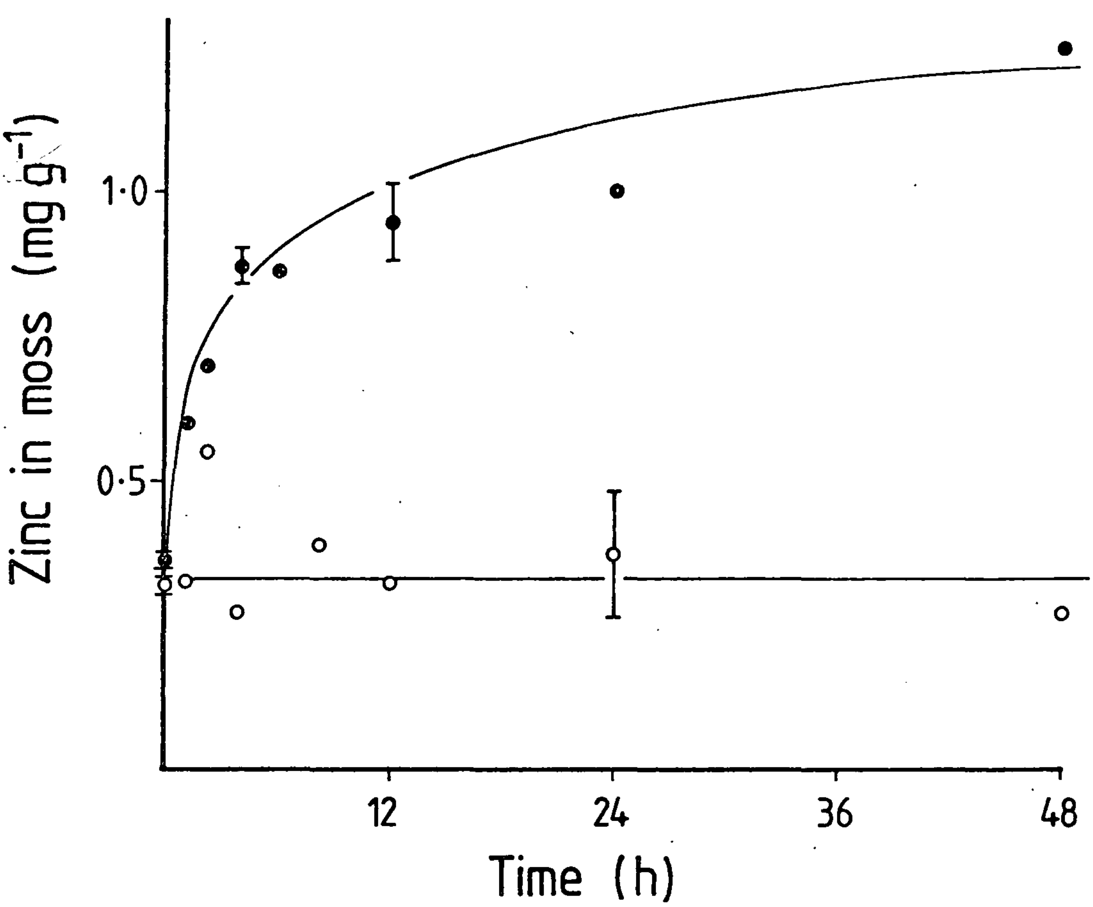
7.312 Effects of Fe-EDTA on zinc uptake

Following the previous results, several experiments were carried out to examine the effects of media-specific factors (as compared with factors common to the environment) which may have inhibited zinc uptake in the laboratory. The first of these tested the effects of the chelating agent EDTA. As this was normally added in a combined form with iron (as $FeCl_3$), experiments were run with and without added iron. In both regimes there was 1.0 mg l^{-1} zinc in the media. Concentrations of zinc in these media after the 48 h accumulation period (Table 7.12)

Table 7.12. Mean (n = 5) filtrable zinc concentrations (mg l^{-1}) in in media after 48 h uptake experiments in different EDTA (+/- Fe) concentrations (µM) (initial zinc concentrations = 1.0 $\pm$ 0.03 mg l^{-1}; * = "normal" EDTA concentration in Durham Chu 10E recipe).

	-Fe		+Fe	
[EDTA]	$\bar{x}$	SD	$\bar{x}$	SD
0.54	0.57	0.02	0.62	0.03
1.08	0.64	0.03	0.67	0.07
5.40	0.71	0.03	0.70	0.07
10.8 *	0.88	0.01	0.82	0.02
54.0	1.19	0.01	1.16	0.01

Figure 7.10. Uptake of Zn by Rhynchostegium in media with 0.4 (open circles) and 1.0 (closed circles) mg l^{-1} Zn, and normal EDTA.



Zinc in moss (mg g^{-1})
1·0
0·5
12
24
36
48
Time (h)

varied considerably with respect to EDTA concentration, but were not different between + or - Fe within a given EDTA level. Accumulation by Rhynchostegium was also affected by EDTA. (Fig. 7.11). Net uptake of zinc in the "normal" level of EDTA was approximately half the maximium. When EDTA was increased further, a net loss of zinc from the moss tips was measured, even in a medium containing 1.0 mg l^{-1} zinc. No significant differences in uptake were found between + and - Fe treatments. Following these results, all subsequent experiments were run with 1.08 µM EDTA, one tenth the normal level.

7.313 Effects of HEPES buffer

The organic buffer HEPES (see Methods for formula) was normally added to the medium to control pH during incubation. Experiments studied the effects of this buffer on accumulation (Table 7.13). Two

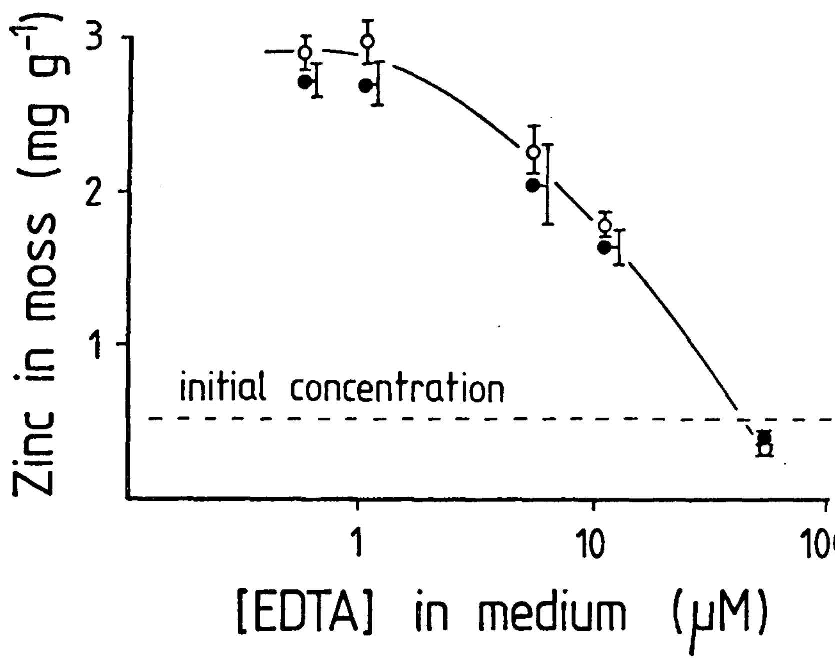
Table 7.13. Concentrations of accumulated zinc in apical tips of Rhynchostegium (mg g^{-1}) after a 48 h incubation (1.0 mg l^{-1} zinc) at varying concentrations of the organic buffer HEPES (mM), both plus Fe-EDTA (1.08 µM) and without Fe-EDTA.

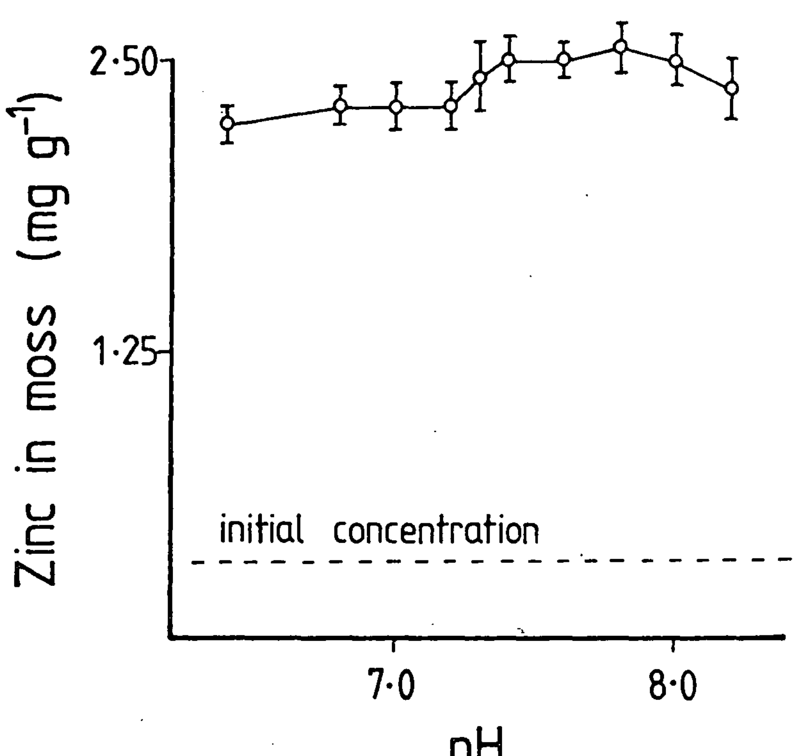
	+(Fe-EDTA)		-(Fe-EDTA)	
[HEPES]	$\bar{x}$	SD	$\bar{x}$	SD
0	2.33	0.10	2.53	0.16
0.5	2.45	0.10	2.54	0.13
1.25	2.43	0.14	2.64	0.10
2.5	2.24	0.06	2.48	0.08
5.0	2.29	0.18	2.43	0.14

groups of treatments were run; with and without Fe-EDTA (1.08 µM) to test any interaction between the two. No significant effects of HEPES were found ($p > 0.05$) within the series of media plus Fe-EDTA. One

Figure 7.11. Effect of increasing EDTA concentrations in media on uptake of Zn by Rhynchostegium (open circles = - Fe, closed = + Fe; Zn in media = 1.0 mg l^{-1}).

Figure 7.12. Effect of pH of the medium on uptake of Zn by Rhynchostegium (Zn in media = 1.0 mg l^{-1}).


Zinc in moss (mg g^{-1})
3
2
1
initial concentration
1
10
100
[EDTA] in medium (μM)


Zinc in moss (mg g^{-1})
2·50
1·25
initial concentration
7·0
8·0
pH

significant difference (18%) was found for 1.25 mM HEPES in the experiment without Fe-EDTA, although there was no evidence of any trend in this set.

7.314 Effects of pH

Prior to examining other environmental effects, an experiment was run to determine whether an "optimum" pH existed for zinc accumulation in the laboratory using this particular medium. HEPES a has near neutral pKa (pH 7.5) and buffers effectively over the range 6.8 - 8.2. Changes in pH and aqueous zinc over 48 h (Table 7.14) indicated several

Table 7.14. Changes in pH and zinc (mg l^{-1}) in media (buffered with 2.5 mM HEPES) in 48 h zinc accumulation period (n = 5; initial [Zn] = 1.0 $\pm$ 0.02 mg l^{-1}).

initial pH ($\pm$ 0.02)	final pH		final [Zn]	
	$\bar{x}$	SD	$\bar{x}$	SD
6.4	6.55	0.06	0.56	0.03
6.8	6.98	0.02	0.50	0.02
7.0	7.19	0.02	0.46	0.02
7.2	7.34	0.01	0.44	0.01
7.3	7.43	0.01	0.42	0.02
7.4	7.48	0.02	0.42	0.01
7.6	7.60	0.01	0.37	0.04
7.8	7.73	0.01	0.35	0.02
8.0	7.82	0.02	0.36	0.04
8.2	7.88	0.01	0.32	0.02

differences among treatments. First, pH tended to shift up in those media which were initially adjusted to below pH 7.60, while those set at pH levels greater than the pKa tended to drift downward. At pH 7.60 no significant change was measured. Concentrations of aqueous zinc remaining in the media after 48 h were less at the higher pH levels, an overall difference in the means of 40% from pH 6.4 - 8.2. While several significant differences were found, final zinc concentrations in media in the range pH 7.6 - 8.2 were not significantly different.

Uptake of zinc by Rhynchostegium (Fig. 7.12) was significantly less only at pH 6.4. The slightly greater accumulation between pH 7.2 and 7.8 was non-significant. The pH of the medium for all subsequent experiments was adjusted to 7.5.

7.32 Experiments using ^{65}Zn

7.321 Initial studies on zinc uptake

The remainder of all laboratory zinc uptake experiments were conducted using ^{65}Zn as a tracer and an unlabelled zinc carrier (for details see Methods). An experiment was first run to test the sensitivity and variability of the method. Prior to the use of ^{65}Zn, 5 or 10 apical tips of moss were required per replicate to achieve the required sensitivity for atomic absorption analysis of a digest. Thus, treatments were replicated in separate flasks. A first experiment examined whether individual tips could be used as replicates and whether variability was kept to a similar level as for pooled tips from separate flasks (Table 7.15). Results indicated variability was usually around 4 - 6% whether tips were used as replicates or if mean values were calculated from separate flasks. An analysis of variance comparing concentrations within and between treatments indicated no significant difference in these two methods. The mean concentration of zinc in tips from one individual flask (no. 3) were significantly greater ($p < 0.05$) than two other flasks in this series. Overall results indicated a low variability and high sensitivity using separate tips for replicates.

Table 7.15. Comparison of variability between zinc accumulation ($\mu g\ g^{-1}$) by individual 2 cm apical tips (n = 5) of Rhynchostegium and pooled results from separate flasks (n = 5), using ^{65}Zn.

flask no.	$\bar{x}$	SD	CV
1	3000	181	6.0%
2	2990	191	6.4%
3	3400	146	4.3%
4	3170	189	6.0%
5	2920	124	4.2%
pooled $\bar{x}$ of 5 flasks	3100	194	6.3%

Changes in the activity of ^{65}Zn standards was examined (Fig. 7.13) by counting the same standards twice, nine days apart. The calibration on each date was a close linear relation, but the slope decreased by about 5%. To account for this, standards were recounted on every experiment.

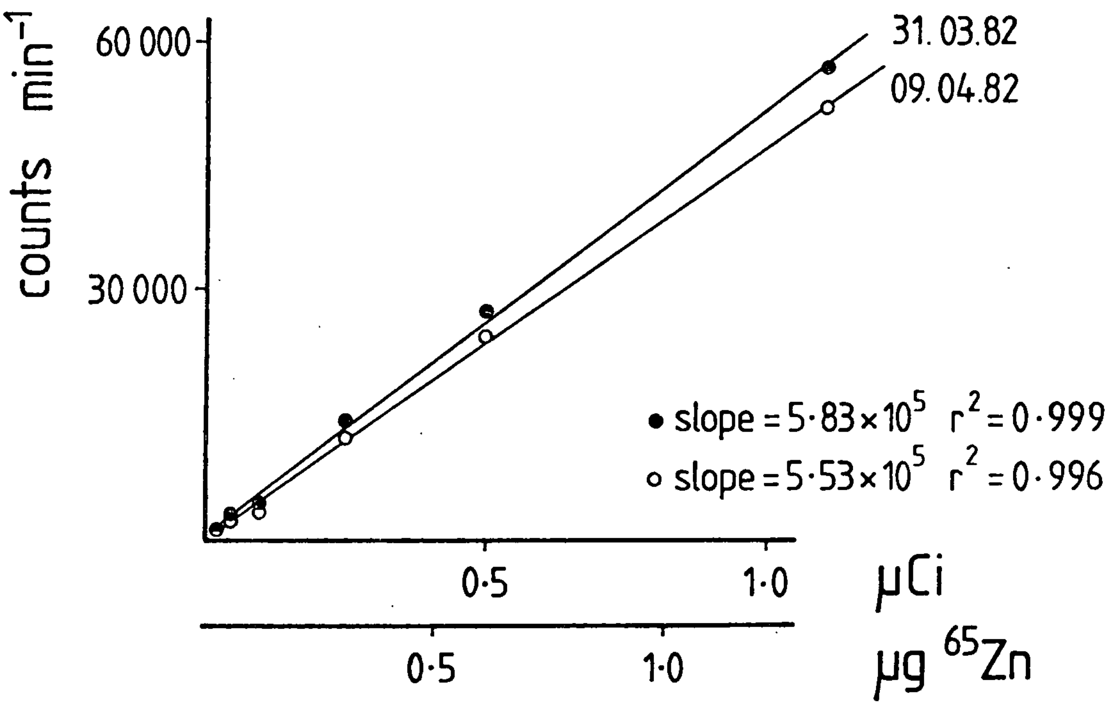
The third preliminary test examined the rate of zinc uptake and the changes in variability during the uptake period (Fig. 7.14). Uptake was found to be fairly rapid, reaching equilibrium within about 8 - 12 h (initial [ ] = 1.0 mg l^{-1}: it was understood that concentrations were not constant in batch culture). There was no increase in variability in zinc accumulated over time, as standard deviations remained similar throughout the experiment. Expresed as a percent, coefficients of variation actually decreased over time.

7.322 Effects of magnesium and calcium

Zinc accumulation was compared over a range of aqueous magnesium concentrations (Fig. 7.15) which included extremes found for Rhynchostegium sites (Appendix 2) and seasonal surveys (chapter 5). A

Figure 7.13. Calibration of ^{65}Zn standards on two different dates.

Figure 7.14. Uptake of zinc in <u>Rhynchostegium</u> over 48 h (Zn in meida = 1.0 mg l^{-1}).


counts min⁻¹
60 000
30 000
31. 03.82
09. 04.82
● slope = 5·83 × 10⁵ r² = 0·999
○ slope = 5·53 × 10⁵ r² = 0·996
0·5
1·0
μCi
0·5
1·0
μg ⁶⁵Zn

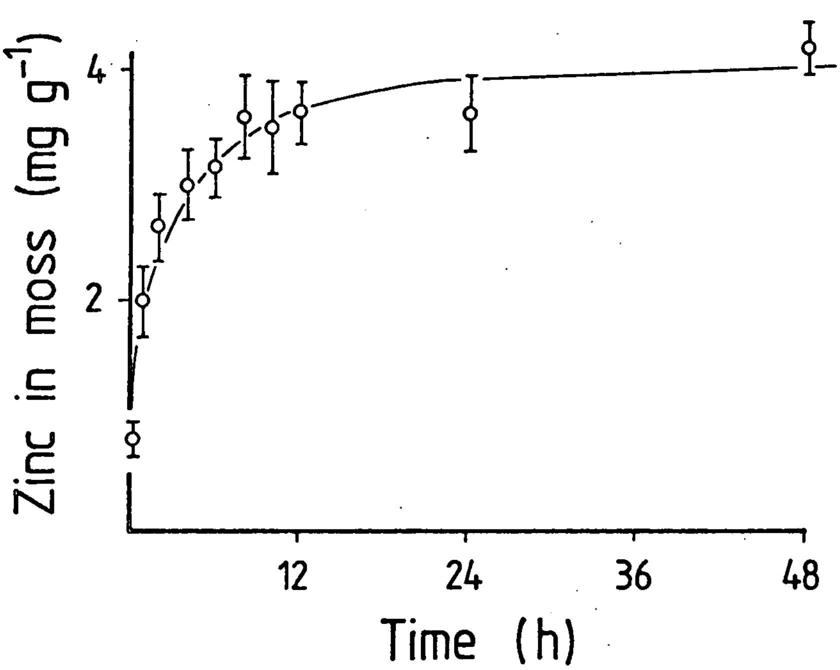
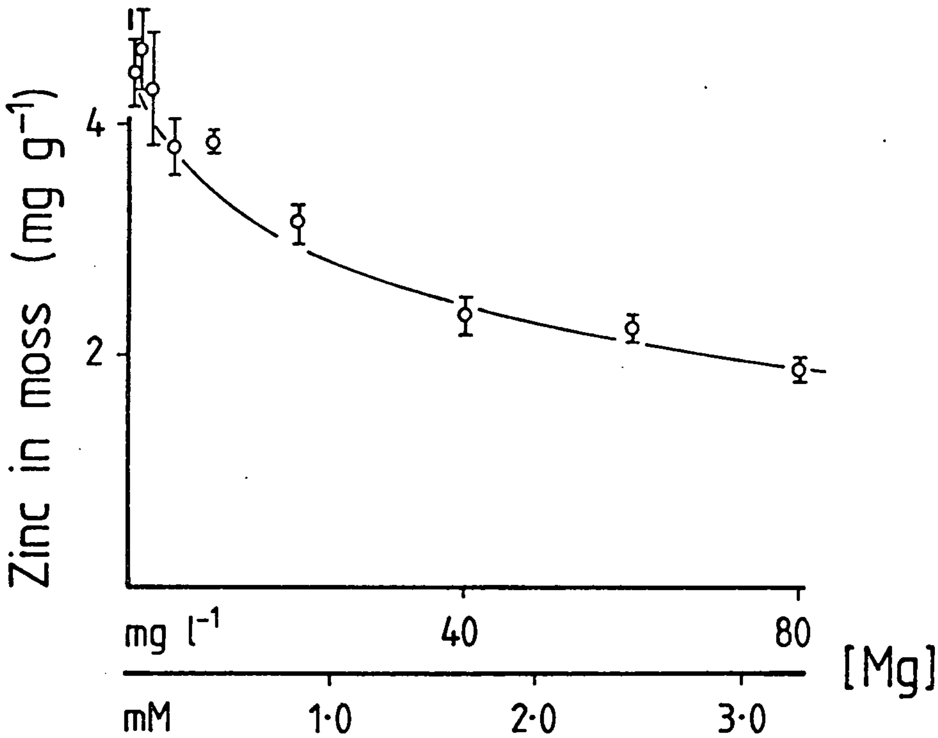
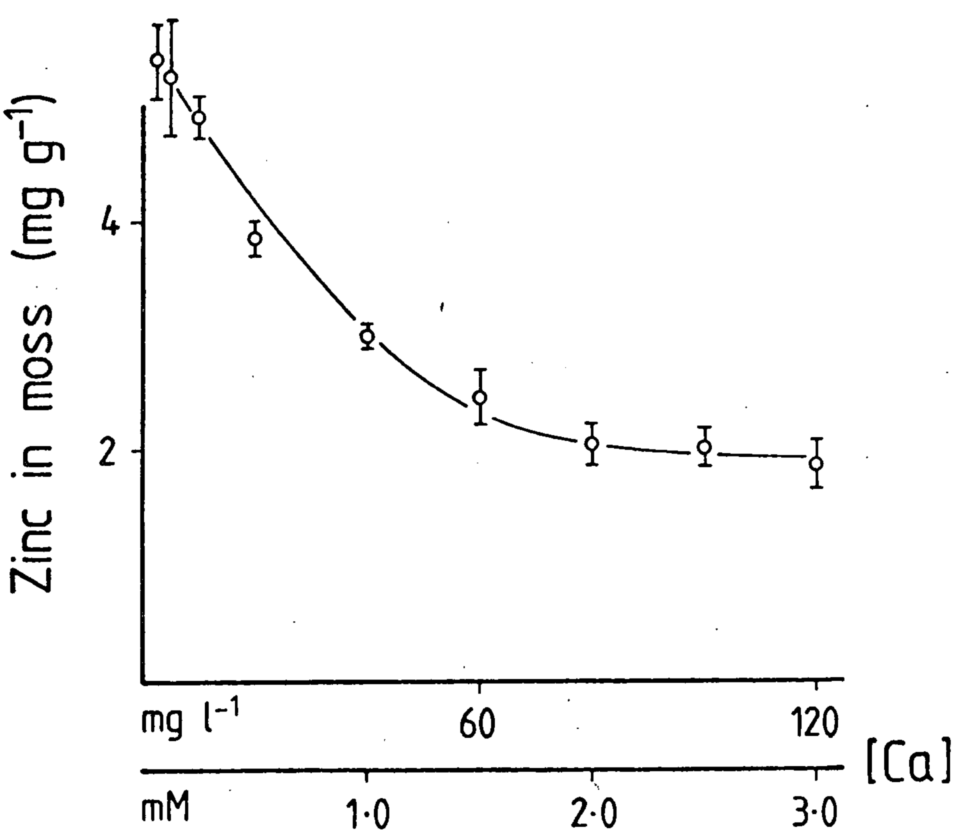

Zinc in moss (mg g⁻¹)
4
2
12
24
36
48
Time (h)

Figure 7.15. Effect of increasing Mg concentrations in media on Zn uptake by Rhynchostegium (Zn in media = 1.0 mg l^{-1}).

Figure 7.16. Effect of increasing Ca concentrations in media on Zn uptake by Rhynchostegium (Zn in media = 1.0 mg l^{-1}).


Zinc in moss (mg g^{-1})
4
2
mg l^{-1}
40
80
[Mg]
mM
1·0
2·0
3·0


Zinc in moss (mg g^{-1})
4
2
mg l^{-1}
60
120
[Ca]
mM
1·0
2·0
3·0

marked reduction was found, particularly between 0 - 20 mg l^{-1}. Several streams previously sampled (Appendix 2) had concentrations > 10 mg l^{-1} magnesium. Zinc uptake was also inhibited by increasing calcium levels (Fig. 7.16). The normal concentration of calcium in this medium (10 mg l^{-1}) was itself somewhat inhibitory. Like magnesium, the most rapid reduction in accumulation was over the lower concentrations, here between 0 - 20 mg l^{-1} calcium, which covers roughly a third of the sites previously sampled (Appendix 2). When compared on a molar basis, approximately 1.25 mM calcium was sufficient to reduce accumulation by half (relative to maximum), while 2.5 mM magnesium was necessary to to produce the same effect.

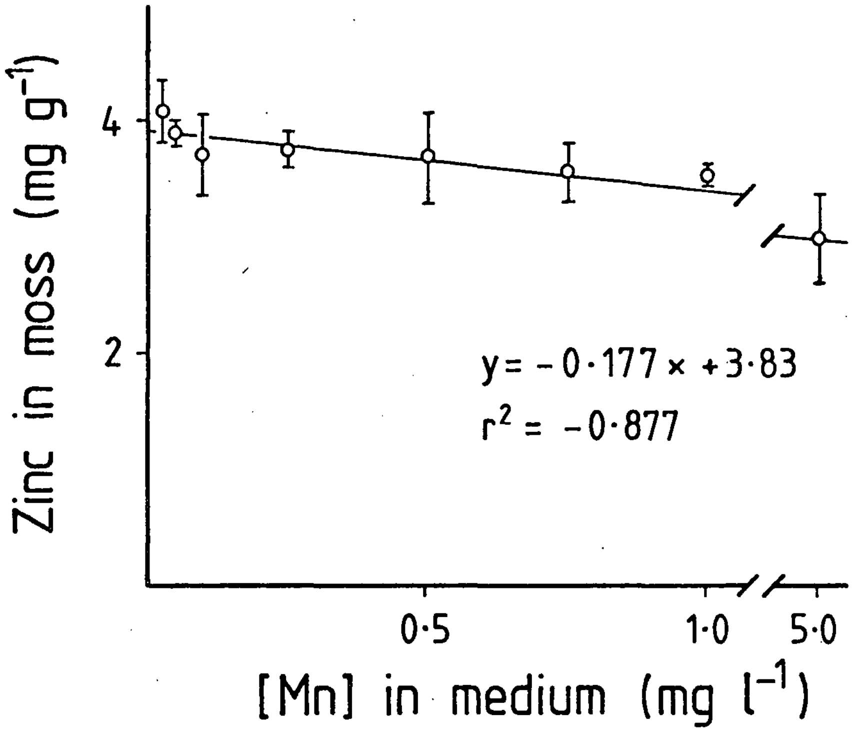
7.323 Effect of manganese

The effect of a broad range of manganese concentrations was tested against zinc accumulation (Fig. 7.17). The decrease over the range 0.025 - 1.0 mg l^{-1} was 13% and at 5.0 mg l^{-1} this reduction was 27%. Although results were somewhat variable, these differences (0.025 v. 1.0 and 5.0 mg l^{-1}) were statistically significant ($p < 0.05$). The linear function of the inhibitory response differed from that observed for the effects of magnesium and calcium.

7.324 Effects of phosphate and nitrate

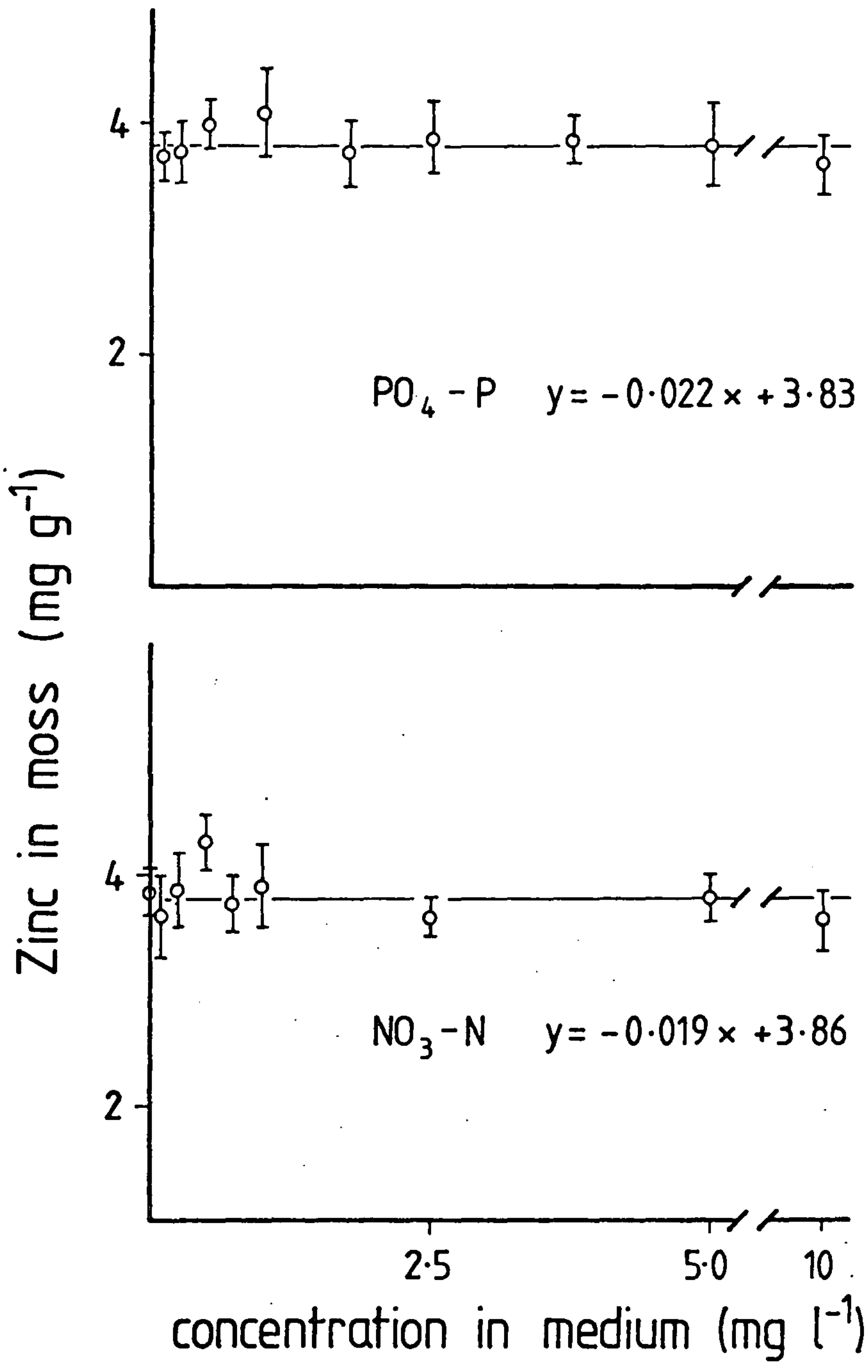
Field surveys and field experiments indicated that some mosses in "nutrient-rich" rivers accumulated less metals (relative to ambient concentrations) than in sites (sections 4.62, 7.22) or times (section 5.23) with lower concentrations. As field data may have been complicated by the correlations among several similarly varying factors, these two nutrients were tested individually (Fig. 7.18). Zinc

Figure 7.17. Effect of increasing Mn concentrations in media on Zn uptake by <u>Rhynchostegium</u> (Zn in media = 1.0 mg l^{-1}).



Zinc in moss (mg g⁻¹)
4
2
y= −0·177× +3·83
r² = −0·877
0·5
1·0
5·0
[Mn] in medium (mg l⁻¹)

Figure 7.18. Effect of increasing PO_4-P and NO_3-N concentrations in media on Zn uptake by <u>Rhynchostegium</u> (Zn in media = 1.0 mg l^{-1}).



Zinc in moss (mg g^{-1})
4
2
PO$_4$-P y= -0·022 x + 3·83
4
2
NO$_3$-N y= -0·019 x +3·86
2·5
5·0
10
concentration in medium (mg l^{-1})

uptake by Rhynchostegium in these regimes was unaffected by either anion, as no significant trend was found. Slopes for both relationships were near zero.

7.325 Effect of silica

The range of aqueous silica concentrations in Rhynchostegium sites from 0.64 - 9.9 mg l^{-1} (section 4.21) was fairly broad. A range which exceeded these extremes was tested (Fig. 7.19). No significant change in uptake was observed ($p > 0.05$) and the slope of the overall response was near zero.

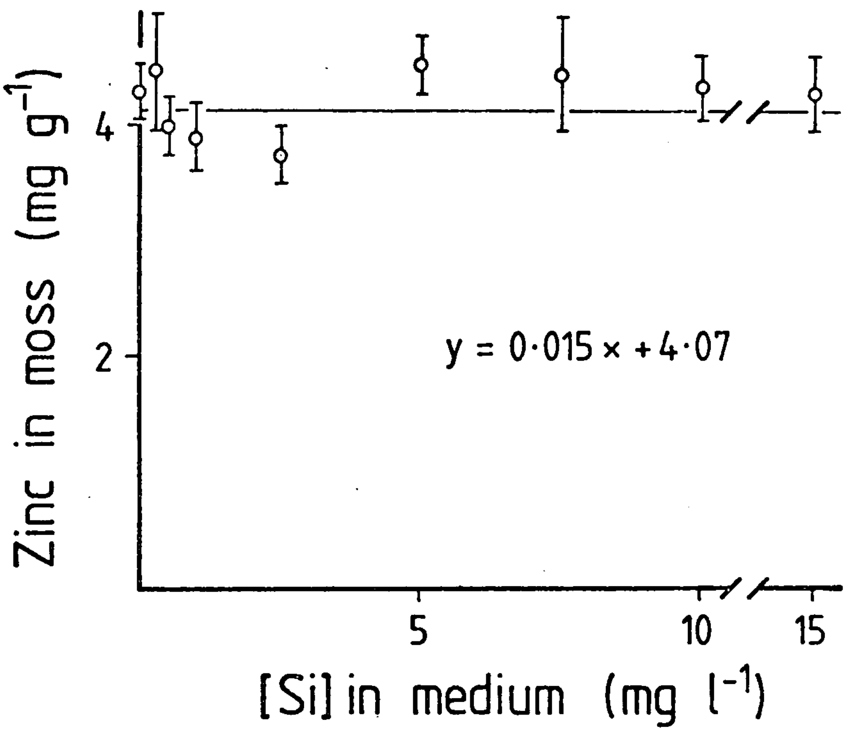
3.326 Effect of humic acids

Concentrations of humic acids were not measured directly in waters sampled during the intensive survey. Optical density (O.D.) measurements of water from this heterogeneous collection of streams were found to be an ambiguous estimate of the degree of colouration (section 4.21). However, many upland streams with brown coloured waters did have high O.D. measurements (e.g. Arkle Beck: 0196-35, -50, -60, -90). Further, the calibration of purified humic acid standards against O.D. (especially U.V. wavelengths) produced highly significant ($p < 0.001$) regression lines (Table 7.16).

Based on the maximum O.D. measurements of upland sites with humates obviously present (chapters 4,5), a range of concentrations were tested against zinc accumulation (Fig. 7.20) in regimes with 1.0 mg l^{-1} zinc. Concentrations of humic acid used were comparable to levels measured directly (as dissolved organic matter) in freshwaters by other workers (e.g. Weber & Wilson, 1975; Cetinkaya et al., 1980). A siginificant inhibition of zinc uptake was found between 0 - 10 mg l^{-1} HA (= O.D. of

Figure 7.19. Effect of increasing Si concentrations in media on Zn uptake by Rhynchostegium (Zn in media = 1.0 mg l^{-1}).

Figure 7.20. Effect of increasing humic acid concentrations in media on Zn uptake by Rhynchostegium (Zn in media = 1.0 mg l^{-1}).


Zinc in moss (mg g^{-1})
4
2
y = 0·015 x + 4·07
5
10
15
[Si] in medium (mg l^{-1})

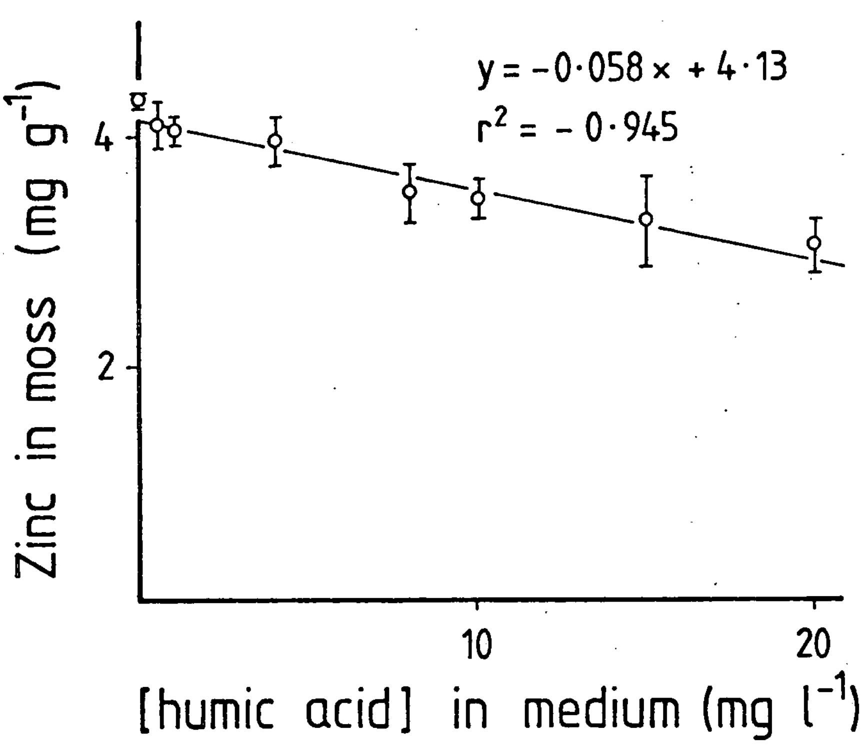

Zinc in moss (mg g^{-1})
4
2
y = −0·058 x + 4·13
r^2 = − 0·945
10
20
[humic acid] in medium (mg l^{-1})

Table 7.16. Relationship between optical density (O.D.) and concentrations (mg l^{-1}) of humic acid (HA) solutions, measured at three wavelengths (nm).

	O. D.		
[HA]	240	254	420
2	0.082	0.078	0.018
4	0.155	0.146	0.034
8	0.326	0.305	0.070
10	0.388	0.368	0.088
20	0.776	0.736	0.182
40	1.575	1.492	0.378
slope	0.039	0.037	0.0095
r^2	0.9998	0.9998	0.9996

0.002 - 0.388 at 240 nm) A concentration of 20 mg l^{-1} HA reduced zinc uptake by 28%. Like manganese, this inhibition followed a linear response with concentration.

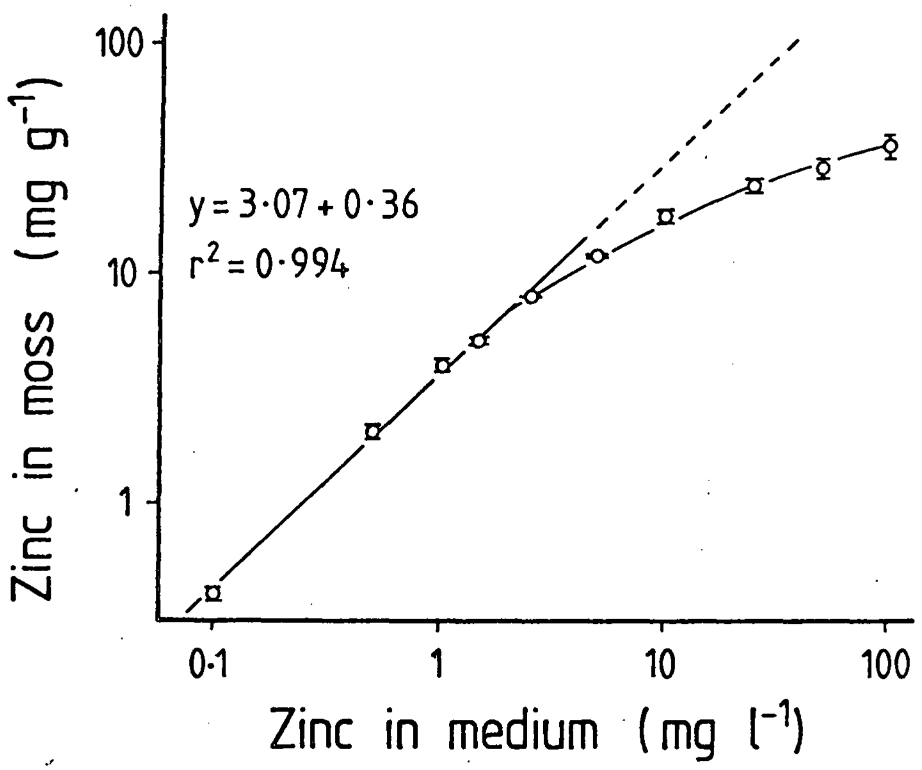
7.33 Other experiments

7.331 Uptake of zinc at different aqueous zinc concentrations

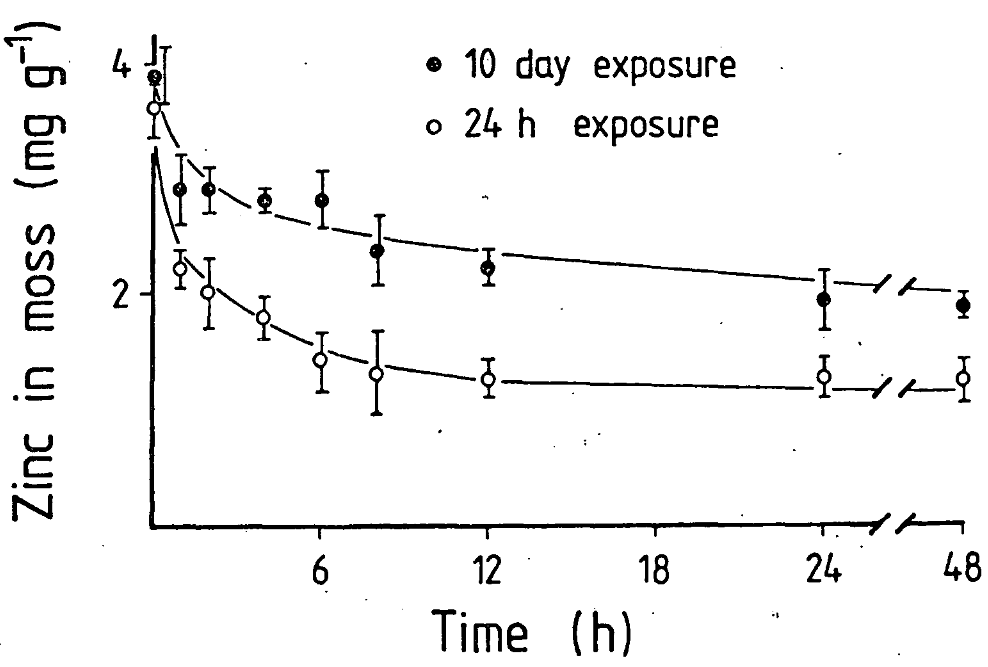
Field surveys (section 4.62) found a strong relationship ($\log_{10}$) between accumulated zinc in Rhynchostegium and aqueous zinc over the range from < 0.006 - 1.62 mg l^{-1}. As it was unknown how this relationship continued beyond the concentrations found in the field, an experiment was conducted using concentrations from 0.10 - 100 mg l^{-1} in the medium (Fig. 7.21). When considered on a log/log basis, this relationship remained constant up to about 2.5 mg l^{-1}. There was a marked departure from linearity (note log scale) at higher concentrations as the plant approached saturation. If the predicted

Figure 7.21. Effect of increasing Zn concentrations in media on Zn uptake by Rhynchostegium (Zn in media = 1.0 mg l^{-1}).

Figure 7.22. Comparison of Zn loss by Rhynchostegium in -Zn media after exposure to 1.0 mg l^{-1} for 24 h and 10 days.



100
10
1
Zinc in moss (mg g^{-1})
y = 3·07 + 0·36
r^2 = 0·994
0·1
1
10
100
Zinc in medium (mg l^{-1})



4
2
Zinc in moss (mg g^{-1})
● 10 day exposure
○ 24 h exposure
6
12
18
24
48
Time (h)

linear relation were extended to 100 mg l^{-1} aqueous zinc, accumulation would have been roughly 300 mg g^{-1} (or about 30%), rather than the 35.2 mg g^{-1} measured.

7.332 Exchangeability

The rate of zinc loss was examined in one field experiment (section 7.2), but because mosses had been in a +zinc stream for an indefinite period prior to the transplant, these plants were theoretically already in equilibrium with ambient concentrations. As uptake has been found to reach a maximum within a relatively short time (this chapter), a comparison was made between rates of zinc loss by bryophytes which had two different periods of accumulation (Fig. 7.22). Apical tips of Rhynchostegium were exposed to 1.0 mg l^{-1} zinc for 24 h and 10 days. After this, tips were briefly rinsed to remove surplus media and then incubated in identical conditions, but without added zinc.

At the beginning of the loss period, concentrations of zinc in the mosses from the two uptake periods were not significantly different (Table 7.17). The initial loss rate (first 2 h) for moss tips exposed

Table 7.17. Comparison of zinc concentrations in Rhynchostegium (mg l^{-1}) during loss period after short (24 hr) and long (10 day) term uptake at 1 mg l^{-1} zinc in media, (differences compared via t-test, n = 5 for each treatment, p = significance).

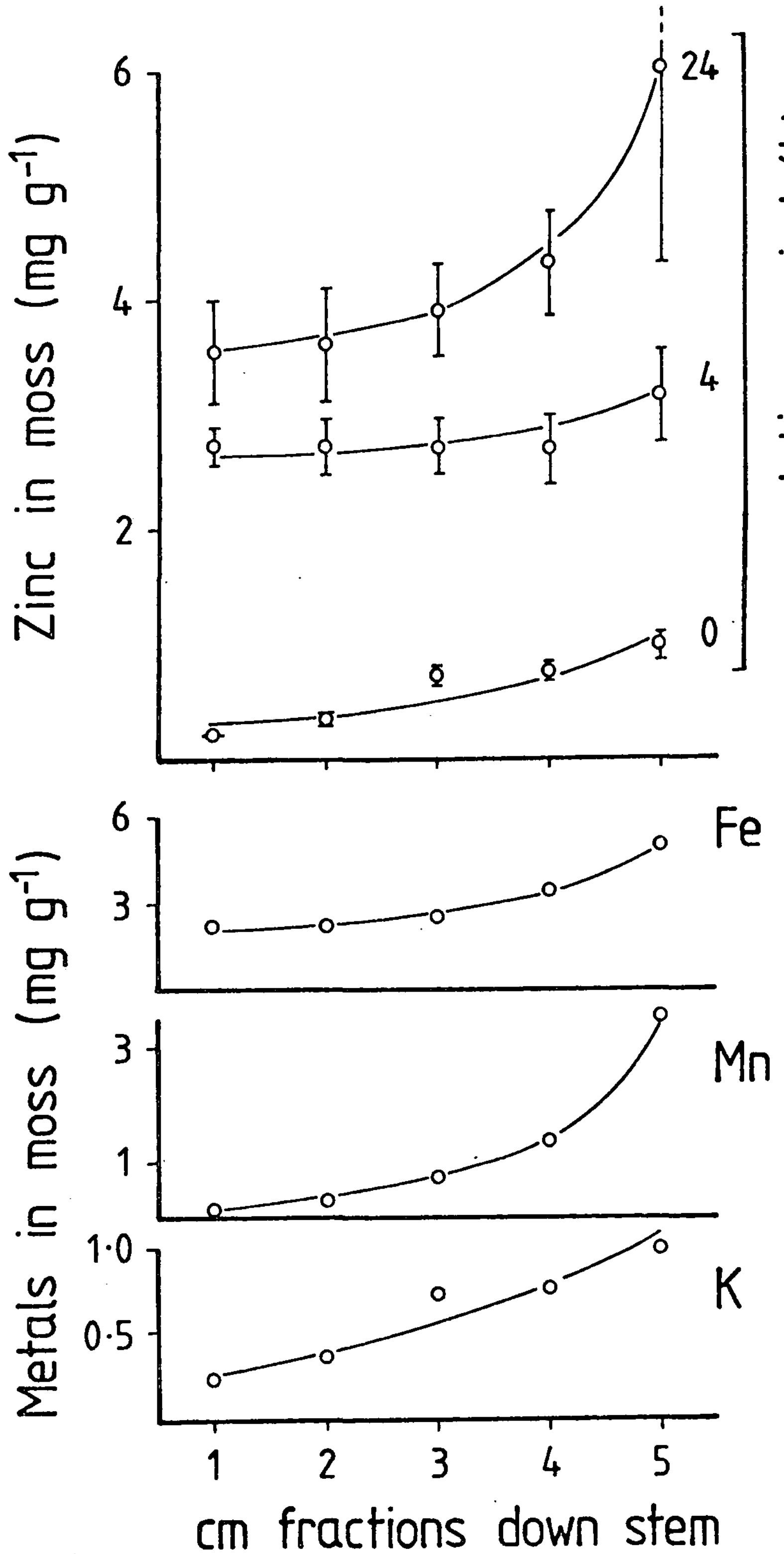
+Zn incubation time	[Zn]: 0h		[Zn]: 24h		[Zn]: 48h		[ZN]: 72h	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
24h	3610	240	1260	180	1230	170	1160	75
10d	3960	330	2270	360	2170	280	2110	180
t-value	-1.93		-18.3		-6.43		-10.5	
result	[24h] = [10d]		[24h] < [10d]		[24h] < [10d]		[24h] < [10d]	
p	> 0.05		< 0.05		< 0.05		< 0.05	

to zinc for 24 h was double that for moss exposed to zinc for the longer period. At 24, 48 and 72 h loss periods, concentrations were significantly greater in moss tips from the 10 day exposure period. Roughly 50% and 65% of the accumulated zinc was lost in in 48 h for the long and short-term treatments, respectively. However, neither reached initial levels (about 0.35 mg g^{-1}) after three days, suggesting that fairly strong binding may have occurred even if exposed to zinc for only 24 h.

7.333 Uptake and localization

Earlier studies (section 7.28) with unmodified populations demonstrated increases in metals, including zinc, along the stems of mosses from both metal-contaminated and uncontaminated streams. These increases were correlated with greater manganese and iron in the lower sections. In this experiment, rates of zinc uptake were compared experimentally along 5 successive cm fractions of Rhynchostegium (Fig. 7.23) to examine whether this was also a rapid phenomenon, as was already shown with 2 cm apical tips. After 4 h, zinc in the first 3 cm fractions increased to roughly 2.7 mg g^{-1}. The lowermost section (cm 5) accumulated about 18% more zinc (difference non-significant) than the first cm section. However, after a 24 h, uptake had increased considerably, such that the fifth cm fraction contained 70% more zinc (mg g^{-1} dry weight basis) than the first cm fraction (difference significant: $p < 0.05$). Thus, the rate of zinc uptake by manganese and iron-rich sections was significantly less rapid, although eventually absolute concentrations became higher.

Figure 7.23. Concentrations of Zn accumulated by successive 1 cm fractions down Rhynchostegium stems after exposure to 1.0 mg l^{-1} Zn for 0, 4 and 24 h, relative to concentrations of K, Mn, and Fe.


6
4
2
Zinc in moss (mg g⁻¹)
24
4
0
accumulation period (h)
6
3
Fe
3
1
Mn
1·0
0·5
K
Metals in moss (mg g⁻¹)
1
2
3
4
5
cm fractions down stem

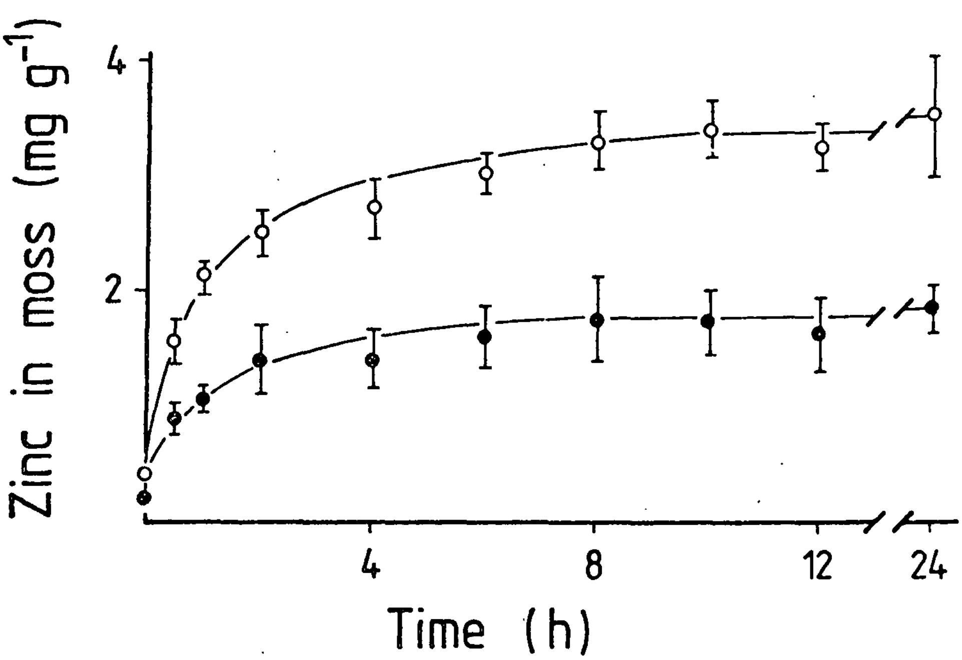
7.334 Uptake by different species

Field surveys (chapter 5) had shown that Rhynchostegium accumulated greater concentrations of metals than other species of aquatic moss, but it was not known whether this also applied to uptake rates. A time course experiment was run comparing Rhynchostegium with Fontinalis antipyretica in 1.0 mg l^{-1} aqueous zinc (Fig. 7.24). Absolute levels in this experiment were clearly greater and uptake apparently more rapid. The initial slopes (Table 7.18) for zinc uptake by Fontinalis was half the rate measured for Rhynchostegium.

Table 7.18. Comparison of initial uptake rate (estimated by initial slope) and net accumulation of zinc by Rhynchostegium riparioides and Fontinalis antipyretica incubated in media containing 1 mg l^{-1} Zn.

species	initial Zn uptake slope	[Zn]: 24h mean	SD
Rhynchostegium riparioides	y = 1740x + 470	3590	630
Fontinalis antipyretica	y = 860x + 260	1840	200

Figure 7.24. Comparison of Zn uptake by Fontinalis antipyretica (closed circles) and Rhynchostegium (open circles) in a medium with 1.0 mg l^{-1} Zn.


Zinc in moss (mg g^{-1})
4
2
4
8
12
24
Time (h)

CHAPTER 8. DISCUSSION

8.1 Introduction

An effort has been made in all parts of this work to coordinate field surveys and experimental studies in examining metal accumulation. Much of the data resulting from these two approaches are complementary, but require a synthesized view of the work as a whole in order to present unified ideas. Discussion will consider fundamental questions arising from the results, as well as practical problems of monitoring heavy metals.

8.2 The ecology of aquatic bryophytes

8.21 Distribution

The present survey (chapter 4) confirmed previous observations that *Rhynchostegium riparioides* was both geographically and ecologically widespread. Watson (1919) states that its ubiquity results from an ability to occur in both siliceous and calcareous districts. However, a good deal more detail is surely needed in understanding the ecological requirements of this species. Few rivers sampled or visited during field surveys lacked *Rhynchostegium* in all sections, which emphasizes the need for more detailed information. The exceptions were smaller streams in softwater catchments where *Scapania undulata* and/or *Hygrohypnum* species were the principal aquatic bryophytes. These observations are not simply explained. Many of such streams (esp. those *Scapania*-dominant) commonly have aqueous calcium between 10 - 20 mg l^{-1} (Whitton *et al.*, 1982); concentrations which were commonly encountered in many streams with Rhynchostegium, such as those in the Mersey catchment and the Lake District (section 4.22: pp. 127-129).

Several aquatic bryophytes and other macrophytes were considerably more restricted. The niche occupied by Rhynchostegium overlaps with upland macrophyte species such as Scapania and Lemanea, as well as lowland species like Fontinalis antipyretica and Cladophora glomerata. However, few streams were ever found that contained both Scapania and Cladophora in the same reach, and few with both Lemanea and Fontinalis antipyretica. The highly restricted distribution of F. squamosa in the intensive survey (section 4.32: p. 149) and reviewed earlier (section 1.422: pp. 44-45) is worth comment. This species was fairly common in northern England west of the Pennines, in upland and lowland sites, although more commonly in softwater. A few upland sites on the eastern side, in upper Swale-/Teesdale (lacking Rhynchostegium and not sampled) did have this species. This moss apparently tolerates at least mild organic pollution (Harding, Say & Whitton, 1981), so there are still too few data available to suggest the cause for such a change.

8.22 Ecological range

The ecological range described for Rhynchostegium was extremely broad. Among sites (chapter 4) and seasons (chapter 5), this species was sampled from a broad range of pH (6.4 - 9.8), nitrate-N (7.5 - 31900 $\mu g\ l^{-1}$) phosphate-P (< 1.5 - 6000 $\mu g\ l^{-1}$) and chloride (5.0 - 355 $mg\ l^{-1}$) levels. By comparison, earlier surveys of rivers with Rhynchostegium (as Eurhynchium) riparioides by Lewis (1974) sampled a more narrow range of these anions (NO_3-N: 10 - 1000 $\mu g\ l^{-1}$; PO_4-P: < 10 - 300 $\mu g\ l^{-1}$; Cl: 10 - 70 $mg\ l^{-1}$). Based on a statistical comparison with chemistries of streams which lacked Rhynchostegium, Lewis (1974) stated that this moss was "significantly restricted" by upper limits of nitrite, phosphate and temperature. Results from the present study suggest that her conclusions were a result of inadequate sampling, rather than real ecological limits. Empain (1974, 1977) has found that Rhynchostegium was ecologically the most widespread in Belgium and northern France, and was particularly tolerant to organic pollution. The abundant growth of R. riparioides (to the exclusion of all other mosses) in the Tanfield sewage effluent (reach 0360-01) certainly supports this. Although a great variety of streams and rivers were sampled for this species, there is no reason to assume the "entire" ecological range has yet been recorded.

Complications arising from attempts to define the "entire" ecological range are illustrated by results for aqueous metal concentrations. Results of the intensive survey (Table 4.02: p. 128) indicate a broad range for most elements, but several measurements from the seasonal survey were well beyond this. For example, the maximum aqueous sodium measured during the intensive survey (T = 89.0; F = 87.8 mg l^{-1}) was roughly half the maximum measured in seasonal surveys (T = 208; F = 188 mg l^{-1}). Concentrations of other metals, including calcium, manganese, iron and zinc extended above and/or below the ranges previously observed during one season. Notable amongst these is zinc, which during the intensive survey was greatest in the River Nent (T = 1.62; F = 1.50 mg l^{-1}). Concentrations greatly exceeded this in the River Team over the year (T = 7.7; F = 6.9 mg l^{-1}). These results all suggest caution should be used when attempting to establish "environmental limits" of aquatic bryophytes based on a limited (even though large) set of data from field collections.

In light of the inadequacies of any finite dataset, it was still possible to establish the maximum likelihood of the realized niche that Rhynchostegium occupies. Green (1971) and Levandowsky (1972) have emphasized the utility of multivariate statistics as an aid in describing the niche in "n-dimensional space" (Hutchinson, 1957: i.e., defined according to a multiple array of ecological factors). A model derived from principal components analysis (pp. 159-164) suggested that the primary factors which are of importance in discriminating amongst the range of potential habitats include a gradient of, for lack of a better single term, "nutrients." These include the anions sulphate, chloride, soluble reactive phosphate, nitrite and nitrate (Table 4.15). Aqueous sodium, magnesium and potassium were also included. These factors are as a group, intercorrelated (pp. 136-140) and thus form a combined gradient. For example, few nitrate- or phosphate-rich sites were poor in chloride. The sites were widely although fairly unevenly distributed along this gradient (Fig. 4.08); 95% of all sites occurred within a more narrow range of intermediate concentrations of these variables. More "outliers" were amongst the nutrient rich extreme than nutrient poor.

The second principal component of the Rhynchostegium niche was a gradient of heavy metals (Zn, Cd, Pb), along which the sites were more evenly spread. A combination of the two principal components of this model (PCA axes 1 & 2) indicated that gaps existed as well. Species such as Scapania undulata fill one space (Fig. 4.08) not occupied by Rhynchostegium, particularly the extremely nutrient-poor streams where concentrations of heavy metals are fairly high (e.g. Zn greater than 1.0 mg l^{-1}). The results suggest that an interaction between these two groups of factors (i.e., extreme heavy metals and low nutrients) may have limited the distribution of Rhynchostegium.

Several physical features of the niche were not measured, in part due to the method of choosing sites and due to sampling design. It has been recognized (Watson, 1919) that many aquatic bryophytes are restricted to larger solid substrates in rivers, and frequently in areas of at least moderate current velocity. These "factors" were ignored because sampling was resticted to such habitats. However, collections of Rhynchostegium, Fontinalis antipyretica and other species have been made (unpublished) from standing water bodies in Scotland (e.g. Loch Lomond, Dubh Lochan, Loch Earn, Lake of Menteith). Published records of aquatic bryophytes are also known in the Lake District (Conniston Water, Ullswater: Welsh & Denny, 1980) and to great depths in lochs in Scotland (up to 12 m: Light & Lewis Smith, 1976). Several of these records were from rocky shorelines subject to wave action, but this is unlikely to be true for deepwater populations. Further, during the intensive survey, Rhynchostegium was found attached to sand as well as to solid substrates (e.g. River Etherow, 0255-25).

One other physical factor which was not considered is the frequency of submergence. It has been shown (Empain, 1974) that this species, while commonly submerged, is also highly resistant to emersion. Plants were found 50 cm above the mean water level in rivers of Belgium and Northern France. This vertical distribution was the most broad of 24 aquatic, subaquatic and non-aquatic species studied. Observations have been made (the author, unpublished) of Rhynchostegium growing abundantly on a wooden water wheel in a tributary of the Gueule, Belgium. Plants were regularly submerged only 5 out of every 30 seconds during rotation.

It is clear from Empain's results (1974, 1977) and the present study that Rhynchostegium occupies a very broad niche. This niche is apparently more diverse than for other mosses suggested as monitors (Whitton et al., 1981b), such as Scapania undulata (Whitton et al., 1982) and Fontinalis antipyretica (Say & Whitton, in press). Nonetheless, results have shown that caution is still needed when attempting to describe a species' environmental requirements and limits.

8.23 Morphological plasticity

It is perhaps to be expected that a species which is ecologically widespread is also morphologically variable. This has been widely observed for terrestrial mosses (Longton, 1979, 1981; Schofield, 1981), but few detailed studies of aquatic species are known, even though floras (e.g. Warnsorf _et al._, 1914; Watson, 1968; Smith, 1978; Crum & Anderson, 1981) and monographs (e.g. Conard, 1959; Robinson, 1962) recognize the extreme variability of aquatic species.

A first step towards an understanding of this phenomenon is the description and classification of a wide number of populations from diverse environments. While terrestrial populations are frequently sampled from numerous locations which are geographically widespread (e.g. Weitz & Heyn, 1981), ecological ubiquity may be sought in aquatic habitats through sampling sites which are physically and chemically diverse. Specimens of _Rhynchostegium_ from the wide range of streams and rivers already discussed, provided an ideal source of material to examine morphological variation. Cluster analysis (section 6.4: pp. 301-304) identified four broad groups of mophological groups, or ecotypes among the 105 populations.

It was clear that the range of morphologies in _Rhynchostegium_ was a response to several environmental factors. This was initially recognized when examining populations with and without organic pollution. Correlations between key variables (esp. ammonia, filtrable reactive phosphate, Na : Ca ratio) support field observations (pp. 297-301); the causes for this are less clear. Morphological plasticity in non-aquatic mosses is often an adaptation to specific environments (Schofield, 1981). It is difficult to recognize whether such variation results from genotypic differentiation (truly separate taxonomic entities) or specific phenotypic responses (adaptability) to local conditions. Present results which have shown considerable intergradation amongst populations and evidence of environmental influences on Rhynchostegium morphology, suggest an adaptable, rather than adapted species. This would clearly be a selective advantage for a perennial species which occupies such a temporally variable habitat (chapter 5). It is known (Rickleffs, 1973) that environmental variability is a strong barrier to evolutionary (and hence genetic) change, and favours species which are capable of different strategies.

Further progress could be gained by following an experimental approach. Reciprocal transplants in particular, can be useful in understanding complex problems of ecotypic differentiation (Clausen et al., 1939). Such experiments are necessary to establish whether genetic differences may exist, as it is known that several species of moss are capable of producing hybrids (Smith, 1978). Studies on aquatic Drepanocladus species (Lodge, 1959, 1960a, b) have shown that within single clones, morphology was strongly influenced by environmental factors, such as submergence, differences in the culture medium and light intensity. Characters which were altered include nerve length and the shape and arrangement of the leaves. However, the shape of the angular cells were not altered by these treatments. These latter characters were also unvaried in field populations of Rhynchostegium.

The present results suggest that reciprocal transplants of robust, large-leaved plants (e.g. Race Fell Burn, 0310-90) with plants with more flaccid texture and smaller leaves (e.g. River Etherow, 0255-25), should induce the reverse traits over a period of time. A lack of morphological change would result in rejection of this hypothesis. An approximate "algal parallel" to *Rhynchostegium riparioides* may be the *Stigeoclonium tenue*, which occurs throughout upland mining streams as well as lowland, organically polluted rivers (Harding & Whitton, 1976). This species has also been shown (Whitton & Harding, 1978) to exhibit considerable morphological variation, which was environmentally induced.

Variability in the morphology of *Rhynchostegium* in various conditions has taxonomic implications as well. As stated earlier (section 2.72: p. 89), one morphological variant was the smaller and narrower-leaved plant from organically polluted waters. This form is confusable with *Amblystegium riparium*, but biometric results have shown that several diagnostic characters remained constant. Most important is the rostrate operculum of the capsule when sporophytes are produced, which is unlike the simpler form in *Amblystegium*. Secondly, leaf arrangement on stems of *Amblystegium* are sub-complanate to complanate, while in *Rhynchostegium* they remain imbricate (overlapping), even when reduced and less robust. The third consistent feature is the presence of rectangular, chlorophyllose angular cells, which differ from the squarer or hexagonal, and pellucid (clear) angular cells of *Amblystegium*. Owing to the variability in *Rhynchostegium*, narrower lanceolate leaves which lack denticulation (occasional in *Rhynchostegium*) should not be regarded as a certain taxonomic character for *Amblystegium*. However, broadly ovate leaves and strong denticulation are positive characters in *Rhynchostegium*. These are lacking in *Amblystegium*. The three diagnostic characters mentioned above may be necessary for identification in some cases, although many rivers where both species are present (e.g. Team, Deerness), no morphological overlap was found. Thus, in the majority of cases the "classical" characters given in floras are adequate for accurate identification.

8.3 Sythesis of field and laboratory metal accumulation results

A number of different approaches were used to study the mechanisms and effects influencing metal accumulation by aquatic bryophytes. Results from field surveys described relationships in situ, from which predictions and hypotheses were produced. In this section, results of field and laboratory experiments are integrated with results from surveys.

8.31 Rates of metal uptake and loss

It has been shown in the field and laboratory that metal accumulation by Rhynchostegium is a fairly rapid process with maxima reached usually within hours. This is also the case for algae (Gutknecht, 1961, 1963; Ahlf et al., 1980) and lichens (Puckett et al., 1973; Nieboer et al., 1976b). Results also show that the regression between ($\log_{10}$) aqueous and accumulated metals follows a fairly straight line. There is evidence of nonlinearity above certain concentrations (5.0 mg l^{-1}) of zinc, however. This saturation plot follows the classic Langmuir isotherm (a rectangular hyperbola), indicating predominantly adsorptive uptake (Bachmann, 1963; Empain, 1977; Nieboer et al., 1976b).

Metal loss was less rapid, as shown through cross-transplants (pp. 307-310) and laboratory experiments (pp. 353-355). These results suggest that the rate of metal loss into the medium or the river is a function of the previous period of accumulation. Plants from the +zinc site in the River Team (0024-20) were theoretically in equilibrium with ambient zinc concentrations for some time prior to the transplant loss period. Loss of zinc proceeded over some days before reaching a stable minimum. Laboratory experiments then compared zinc loss in plants which had been in a +zinc medium for 24h v. 10 days. Those from the shorter accumulation period lost this metal more rapidly in -zinc media, even though zinc concentrations in plants from the two treatments were insignificantly different at the start of the desorption period.

Rapid uptake of zinc by Cinclidotus danubicus has also been observed (Empain, 1977). Desorption, after a 48 h uptake period was slower and only 20 - 25% of the total zinc was lost after reaching a stable equilibrium. A slow release of accumulated metals has also been shown in Ulva, Porphyra (Gutknecht, 1963) and Laminaria (Bryan, 1969). These and the present results for Rhynchostegium are in agreement with the model described by Pickering and Puia (1969), which states that zinc uptake occurs in three stages. The first is a short, rapid period ("exchange adsorption") of uptake into the Donnan free space, where the metal remains readily exchangeable. This is followed by two slower processes where the metal is taken up within the protoplast and into the cell organelles. The latter compartments apparently isolate potentially toxic metals and function as a barrier to rapid exchange (Pickering & Puia, 1969), which in turn restricts the rate of desorption.

It was found that 9 - 12% of ^{65}Zn accumulated by Fontinalis antipyretica after a 7 day incubation was readily exchangeable in aqueous or alcohol-soluble washes, as compared with cell wall fractions (Burton & Peterson, 1979b). The authors suggested this was evidence of the immobilization of zinc through binding by the cell walls. Lead was apparently also capable of binding to cell walls in Grimmia donianna, although it failed to penetrate the cytoplasm (Brown & Bates, 1972). While no direct observations (e.g. electron microscopy, x-ray microanalysis) are known for such cellular localization of zinc in aquatic mosses, it has been shown to localize in the nuclei of cells from barley roots exposed to ^{65}Zn (De Fillipis & Pallaghy, 1975). Zinc can also penetrate the cell membrane of several types of tissue in the common mussel, *Mytilus edulis* (George & Pirie, 1980) and has been observed in membrane-bound vesicles within the cytoplasm. Inclusions of lead within the cytoplasm (Gullvag *et al.*, 1974) as well as the chloroplasts, mitochondria and nuclei (Skaar *et al.*, 1973) has been observed in *Rhytidiadelphus squarrosus*. Lead localization in *Stigeoclonium* has been observed (Silverberg, 1975), principally on cell walls and within cytoplasmic vacuoles. Ultracellular inclusions of lead have also been identified in *Potamogeton pectinatus* (Sharpe & Denny, 1976).

Assuming that binding of metals on cell walls in mosses occurs a short time after the initial rapid uptake period (Pickering & Puia, 1969; Burton & Peterson, 1979b), loss should proceed more slowly than uptake. This has been demonstrated experimentally for *Rhynchostegium* and numerous other species. This mechanism is typical of two or more compartment diffusion models, unlike that for simple (non-biological) ion exchange membranes or resins (Hope, 1971).

8.32 Factors affecting accumulation

Much of the initial work in the present study on factors affecting metal accumulation was carried out using a multiple regression model (section 4.62: pp. 167-191). This revealed chemical variables which were significantly related (+ or −) to increased metal accumulation by moss populations *in situ*. The principal factor influencing the accumulation of zinc, cadmium, barium and lead (apical tips and whole plants) in field populations of Rhynchostegium was the concentration of the respective metal in streamwater. Similar statistical techniques have shown that for Scapania undulata (Whitton et al., 1982) this was true for zinc, but not for the accumulation of cadmium or lead.

Several other variables were selected by multiple regression, but a few specific factors correlated consistently (+ or - effects) with accumulation of most metals. Water chemistry variables included aqueous magnesium (-), calcium (-), manganese (- or +), iron (-), phosphorus (-), ammonia (+) and nitrate (+). Concentrations of potassium (-), calcium (+), manganese (+) and iron (+) in the moss were also included, particularly for whole plants.

In field experiments, moss transplants (section 7.22: pp. 310-314) into two rivers with similar aqueous zinc resulted in significantly greater accumulation in the stream with less aqueous magnesium, calcium and phosphate. It was possible to design an experiment testing the effects of these combined variables in the field, but not whether one specific factor was principally involved. Laboratory experiments (sections 7.322-7.324: pp. 338-351) have shown that both magnesium and calcium significantly inhibited zinc uptake; on a molar basis calcium was roughly twice as inhibitory. However, none of the anions tested (PO_4-P, NO_3-N, Si), over a very wide range of concentrations, had any significant effect. This suggests that their influence on field populations, at least for zinc may not be great. Indeed, significant correlations from field data are not proof of cause and effect relations; many entirely independent variables may themselves be intercorrelated.

Field results for the accumulation of zinc by populations of

Lemanea (Harding & Whitton, 1981) suggest the negative influence of calcium and/or magnesium (likely both) as well. Phosphate did not vary sufficiently in the sites where Lemanea was collected to be considered. It has been shown that magnesium (Cushing & Rose, 1970) and calcium (Pickering & Lucas, 1962) inhibit the adsorption of radionuclides by algae. The uptake of zinc by the moss Hylocomium splendens was inhibited more strongly than was the uptake of other heavy metals (Co, Ni, Cd, Pb), by the presence of high concentrations of sodium, magnesium, potassium and calcium (Ruhling & Tyler, 1970). Magnesium and calcium have also been shown (Bachmann, 1963) to be more effective in reducing zinc uptake than other ions, such as sodium and potassium.

Although no experiments have been carried out on the effect of magnesium or calcium on the accumulation of other heavy metals by Rhynchostegium, they have been shown (Kinkade & Erdman, 1975) to inhibit cadmium uptake in several aquatic plants and animals. Calcium can also inhibit uptake of lead by grasses (Garland & Wilkins, 1981) and nickel by the serpentine plant Alyssum serpyllifolium (Brooks et al., 1981). Although it may appear that magnesium and calcium may inhibit metal accumulation in general, calcium had apparently no effect on zinc uptake by the yeast Candida utilis (Failla et al., 1976) and in one study (Welch, 1973) calcium was found to stimulate vanadium uptake in barley.

It has been suggested (Whitton & Say, 1975) that factors in the environment which affect the toxicity of metals may affect accumulation similarly. Although results for magnesium and calcium support this theory, neither phosphorus or nitrate had any effect on zinc uptake by Rhynchostegium. It is possible that anions act differently on the uptake of metals in various organisms or particular elements. For example, phosphate reduced the uptake of zinc by Thalaspi alpestre

(Ernst, 1968) and the alga Scenedesmus obliquus (Keulder, 1975). In contrast, uptake of zinc by rice (Dogar & Van Hai, 1980) was stimulated by increasing phosphate-P, up to 300 μM, but was supressed at higher concentrations.

A signficant positive effect of nitrate on copper uptake has been demonstrated (Jarvis, 1981) with grasses in flowing solution cultures. Antonovics et al. (1971) have explained that factors relating to the toxicity of metals do not always follow for accumulation, as tolerance in some organisms is derived through a reduced uptake of metals, while others actually accumulate greater concentrations than their non-tolerant counterparts.

In the present study a slight (< 10%) increase in accumulation of zinc over the pH range 6.4 - 8.2 (approximating the range for most Rhynchostegium sites) was found; uptake was significantly different only at pH 6.4. This is inconsistent with most experimental results (Gutknecht, 1961, 1963; Bachmann, 1963; Francis & Rush, 1974; Wainwright & Beckett, 1975; Nieboer et al., 1976a, b). Ahlf et al. (1980), however, emphasize that pH effects in laboratory studies of metal accumulation vary considerably when EDTA is included in the medium. Indeed, of the studies cited above, both positive and negative effects were found with increasing hydrogen ion concentration. EDTA itself exerted a considerable influence on zinc uptake in the present study. Chelation of zinc by EDTA usually forms highly stable complexes, which are known to be influnced by pH and other factors (Raspor et al., 1981).

Humic acids are common in freshwaters (Wetzel, 1975) and in aqueous systems the observed yellow-brown colour may be associated with both fulvic and humic fractions, although humates tend to predominate at higher pH values (Kerndorff & Schnitzer, 1980). In laboratory experiments with Rhynchostegium, these substances significantly reduced the accumulation of zinc. Giesy (1976), using dialysis experiments, has demonstrated that humic acids reduce iron availability to Scenedesmus obliquus directly through binding, thus inhibiting accumulation. Present experiments show an important influence of humates on zinc accumulation, although presently it is not possible to generalize about their effect in nature, as the formation of metal:humic acid complexes are strongly influenced by several factors, including pH (Kerndorff & Schnitzer, 1980), calcium and alkalinty (Wilson, 1978). Much useful information could be gained on the mechanisms of metal accumulation from further studies on the influence of humates and their interaction with other complexing substances.

That there is greater agreement and understanding of the effects of certain cations on metal uptake is not surprising. Lichens (Brown & Slingsby, 1972; Puckett et al., 1973; Wainwright & Beckett, 1975) and bryophytes (Clymo, 1963; Ruhling & Tyler, 1970; Molchonva & Bochenina, 1980) have been widely recognized as biological cation exchangers, hence the interaction between the uptake and release of complementary cations (Nieboer et al., 1976b). While it is clear that the ion exchange model is useful for describing properties of metal uptake, it does not fully explain the marked non-linear relationship between increasing aqueous magnesium or calcium and decreasing zinc uptake in Rhynchostegium. It has been found for a variety of bryophytes, including Fontinalis antipyretica, that calcium and magnesium do not act as "osmotic buffers" the way in which potassium does (Brown & Buck, 1979). The sorption of copper by Hylocomium splendens was apparently not entirely referrable to simple ion exchange properties (Ruhling &

Tyler, 1970). Instead, calcium and other cations may act to inhibit uptake through competition for binding sites, as is known for Fe-EDTA (Stumm & Morgan, 1981).

8.33 Localization

The principal method used to study the physical localization (as compared with subcellular localization) of metals in aquatic bryophytes was to examine changes in metals down the length of the stem. Increases in concentrations of certain metals, particularly manganese, iron, zinc and lead (section 7.28: pp. 324-329) support predictions of regression analyses (section 4.62: pp. 167-191), that heavy metal accumulation by populations of *Rhynchostegium* was influenced by manganese and iron in the mosses. Serial fractions of *Fontinalis antipyretica* (Whitton *et al.*, 1981a) and *Scapania undulata* (Whitton *et al.*, 1982) show similar patterns. Whether this is strictly the result of coprecipitation of metals, as has been shown for sediments (Jenne, 1968; Robinson, 1981) is not clear. A similar increase (on a dry weight basis) of several metals (Mn, Fe, Zn, Pb) has been observed in *Hylocomium splendens* (Ruhling & Tyler, 1970) and species of *Sphagnum* (Pakarinen, 1978). These authors found a parallel reduction in sodium and potassium down the stem, suggesting at least some influence of cation exchange (in addition to coprecipitation). There is some evidence of potassium efflux in *Rhynchostegium*, but the studies on *Fontinalis* and *Scapania* did not examine this.

The importance of the manganese- and iron-rich fractions were also examined for rates of metal uptake. It was found that ultimately (after 24 h) these lower fractions accumulated far greater concentrations of zinc, but that uptake was slower than for the apical fractions. Uptake by lower fractions proceeded slowly perhaps because ion-exchange sites of the cell were covered by these inorganic precipitates. Through various solvent extraction procedures and sonication (K. Satake & P. J. Say, pers. comm.) it has been shown that metals in these lower fractions are nonetheless very tightly bound.

Significantly greater amounts of several metals were found in leaves of Rhynchostegium than in stems (dry weight and absolute basis: pp. 323-324), especially manganese and iron. A simple explanation would be that leaves, which are a single cell thick, would be capable of greater adsorption than stems, which are several cells thick. However, Hebant (1977) has shown that many mosses (including R. riparioides) possess primitive, but functional conducting systems, which presumably are capable of translocating soluble metals throughout the moss. The more homogeneous distribution of potassium, calcium and zinc, compared with lead and manganese, supports several studies which suggest that lead remains bound to the cell wall surface (e.g. Brown & Bates, 1972; Brown & Slingsby, 1972), while zinc may penetrate the cell membrane (e.g. De Fillipis & Pallaghy, 1975; Burton & Peterson, 1979b) along with apparently mobile cations like potassium.

8.34 Interpopulation differences

Although comparisons of metal accumulation have been made between different species of aquatic bryophyte (McLean & Jones, 1975; Empain, 1976a, b; Burton & Peterson, 1979a; Say et al., 1981) little is known about differences among different populations of a single species. The approach for such studies which has attracted most interest has been to examine the uptake of metals in heavy metal tolerant and non-tolerant populations (Antonovics et al., 1971).

A population of Rhynchostegium was isolated from a zinc and lead contaminated stream ("High Crag Burn," 0101-05), from which a known zinc tolerant strain of Hormidium rivulare had been isolated (Say et al., 1977). Zinc uptake (Fig. 7.05: p. 319) was not significantly different from another population isolated from an uncontaminated stream. However, uptake of lead (Fig. 7.06: p. 319) was significantly higher (more than twice) in the presumed tolerant moss. Similar studies have examined the accumulation of one metal only (e.g. Cu: Butler et al., 1980; Zn: Harding & Whitton, 1981; Pb: Brown & Bates, 1972), even when multiple metal tolerance might reasonably have been suspected in plants from mining polluted environments. The reason for a difference in the uptake of lead but not zinc by the two Rhynchostegium populations is not obvious, particularly in that many zinc tolerant higher plants accumulate more zinc than non-tolerant populations (Turner & Marshall, 1971, 1972; Baker, 1978; Brooks et al., 1981). However, Baker (1981) has explained that "excluder" and "accumulator" strategies are known to exist. It may be that in Rhynchostegium opposite strategies are used for zinc and lead. Although much of the lead may have adsorbed to the cell wall, some may be tolerated internally if localized within pinocytotic vesicles, as in Stigeoclonium (Silverberg, 1975) and Hylocomium splendens (Gullvag et al., 1974).

Differences in zinc uptake by different populations of moss all isolated from low metal (e.g. < 0.05 mg l^{-1} Zn) streams requires explanation. No other studies are known which have made similar studies with any kind of aquatic plant. Accumulation by the five fell into two distinct groups, with no apparent differences in their previous experience of heavy metals. In light of the insignificant relation between enrichment ratios and morphology (section 6.4: p. 305), it is unlikely that differences in growth form played a part. Differences in microhabitat during the uptake period are also unlikely, in that a specific experiment run simultaneously showed no significant difference in zinc uptake by mosses in a pool and a riffle. Harding and Whitton (1981) also found no significant difference in concentrations of heavy metals in Lemanea populations collected from these two regimes. Of the two groups of Rhynchostegium populations, the "high accumulators" (Thornhope Beck and River Browney populations) were found to contain much greater concentrations of manganese (Appendix 5: pp. 430-432) than the "low accumulators" (no obvious trends for Na, K, Ca, or Fe), and may have had some effect.

These and the previous interpopulation studies present interesting problems worth studying further. For example, it would be useful to know whether significant differences in metal concentrations between certain populations in a single stream continue over longer periods. Such a result might suggest biological and/or genetic causes, while strictly chemical differences (e.g. Mn in moss) would change in time.

8.35 Seasonality

An appreciable amount of seasonal variation was observed for metal concentrations in populations of aquatic byophytes (chapter 5). Metals showing the least variation overall were magnesium and calcium, even though aqueous concentrations of these elements were perhaps the most obviously seasonal (low concentrations in autumn-winter), and at all sites studied. Many of the differences in concentrations of zinc, cadmium, barium and lead in mosses (Amblystegium riparium, Fontinalis antipyretica, Rhynchostegium riparioides) over one year could be accounted for by two major causes. The most important factor (based on numbers of significant correlations) was the temporal flux of the aqueous metal concerned. A concomitant change in accumulated metal is expected; in fact, it is a positive advantage from the monitoring point of view. The second factor related to seasonal differences was the variation in physical and chemical factors, which themselves may affect metal accumulation. As noted above, aqueous magnesium and calcium, which have been shown to affect zinc uptake, followed obvious seasonal trends. One example of this was observed in the River Wear (0008-65); aqueous lead remained generally low, while accumulated lead (Fontinalis and Rhynchostegium) followed an inverse seasonal pattern with aqueous calcium. In general, seasonal results satisfy the predictions of the multiple regression analyses for between-site differences in metal accumulation.

Strictly seasonal or temporal effects (e.g. plant growth) were apparently less important to metal accumulation. Few signficant correlations were found in a lagged time-series analysis of plant:water cross-correlations (section 5.4: 276-283). As much of the variation found was attributable to factors previously recognized (e.g. calcium differences), it is believed that the importance of seasonal variation in itself was not as great as was first suspected at the outset of this study.

Whether age- or season-dependent growth forms and growth rates may be important for aquatic bryophyte is unkown, although the present data gives no evidence of this. Mouvet (1980) has observed significantly different slopes of accumulated:aqueous chromium in field populations of aquatic mosses, when sampled on three different months. Although specific factors were not tested, the author states that differences in accumulation were likely the result of seasonal variation in physicochemical conditions of the rivers sampled. Distinct, seasonal patterns in the development of bryophytes is well documented (Longton, 1980), but are largely restricted to phenological changes in gametangial and sporophyte production. Clearly, the gametophyte (leafy stage) of bryophytes, compared with the dominant phases of angiosperms and many seaweeds, is morphologically less variable over the year. For example, no populations were observed to alternate between a predominantly protonemal growth form and the leafy stage. Several aquatic mosses are in fact known (Watson, 1968; Smith, 1978) to only rarely reproduce sexually (e.g. Fontinalis antipyretica), presumably continuing through the year by vegetative means.

While it must be assumed that some specific biological or physiological changes in aquatic bryophytes occur seasonally, the relevant studies which are known (Bodin & Nauwerck, 1968; Clymo, 1970; Glime & Acton, 1979; Sanford, 1979; Priddle, 1980) have not considered the importance of such processes to metal accumulation or ion exchange. The present results of seasonal surveys on metal accumulation satisfy the predictions of the multiple regression models (pp. 267-291). Evidence is given by contrasting results from different streams. In rivers where clear seasonal trends were observed (e.g. West Allen and Wear), significant correlations with several other water chemistry variables, such as calcium, were found with variations in metal accumulation. In a river lacking regular seasonal changes in water chemistry (e.g. Team), accumulation followed closely aqueous metal concentrations. Further useful information on the importance of seasonal changes in growth and phenology alone would be gained through an intensive study of aquatic bryophytes in a spring (e.g. for algae: Patterson & Whitton, 1981), where physicochemical fluctuations are at a minimum.

8.4 Considerations for monitoring metal pollution

It should be clear from earlier parts of the discussion that many problems which have been studied related to fundamental questions regarding aquatic bryophytes, while others were concerned with the use of bryophytes as monitors of metal pollution. Consideration here will be given to the practical side.

8.41 Evidence of contamination

After early studies demonstrated that aquatic organisms could accumulate potentially toxic heavy metals, plants were soon being recommended widely as as "indicators" or "monitors" of pollution (e.g. Kirchmann & Lambinon, 1973; Dietz, 1973; Whitton *et al.*, 1981b). It is not always clear, however, what represents a "significant increase" of a metal in a plant, as compared with so-called "background" concentrations (Hargreaves, 1981). Frequently used terms, such as "contaminated," lack meaning unless discussed in relation to uncontaminated environments. This is probably the cause of several misunderstandings in the literature (e.g. Rushforth *et al.*, 1981), where certain species have been designated as indicators of "high" or "low" concentrations of heavy metals, in waters lacking any evidence of pollution.

In the present study, specific "baseline" concentrations of several metals (Cr, Zn, Cd, Ba, Pb) in *Rhynchostegium* (apical tips) were estimated (Table 4.13: p. 154). Concentrations above these may be considered evidence of contamination. Critical studies could provide useful information by including more data for metal concentrations of potential monitoring species in waters presumed to be uncontaminated. A biologist not familiar with problems of heavy metals or a water authority chemist may have little experience in deciding whether, for example, 5000 $\mu g\ g^{-1}$ zinc in a moss is to be considered "high" or "low."

8.42 Sensitivity

The concentration of a metal in Rhynchostegium was found primarily to be a function of the aqueous metal concentration. Therefore, comparisons with different plants in their ability to accumulate metals require a standardized index or value relative to aqueous concentrations. The most common index used is the enrichment ratio (Brooks & Rumsby, 1965). This ratio have been given several other names, including enrichment factor (Dietz, 1973), accumulation factor (Empain et al., 1980), concentration ratio (Harvey & Patrick, 1967) and concentration factor (Hutchinson & Stokes, 1975; Seeliger & Edwards, 1977). Aside from the confusion of names, this theoretically allows simplified comparisons between different species or between different environments.

Marked differences in the enrichment ratios of heavy metals in Rhynchostegium (apical tips) were found between elements (pp. 165-167), averaging from roughly 2400 (Ni) to over 94 000 (Pb). The maximum single enrichment ratio for apical tips was measured for lead, at 2.5 million. Sensitivity by this index, was found on average to be greater in whole plants than apical tips for all metals considered (Co, Ni, Cu, Zn, Cd, Ba, Pb). These results, when compared with results for other aquatic bryophytes (Empain et al., 1980), seaweeds (Seeliger & Edwards, 1977), and a variety of organisms (Whitton & Say, 1975), suggest that Rhynchostegium has considerably greater sensitivity than many aquatic plants and animals.

Few other studies are known which have examined enrichment ratios of a single species from as many diverse sites. One extremely high value (e.g. Pb) may alter this "average" considerably. Present results have shown that coefficients of variation in enrichment ratios for a single species commonly exceed 100%. Further, the ratio itself has been shown to be inversely related to aqueous metal concentrations, not only for Rhynchostegium but also for Lemanea (Harding & Whitton, 1981). It has been shown in comparisons between a wide variety of organisms (Jinks & Eisenbud, 1972; Kneip & Lauer, 1973) that this ratio varies considerably due to both environmental and physiological factors. Jinks and Eisenbud (1972) have explained that in using such an oversimplified index, some authors have even introduced errors by the use of values from the literature for "average" elemental concentrations in waters, rather than basing it on water from which the organism was sampled.

In light of these problems, it is suggested that the enrichment ratio be replaced with the slope and intercept of the linear regression between metal accumulation and metals in water. This index is unambiguous, statistically more robust than a ratio (Green, 1979) and biologically more meaningful. The intercept provides information as to the minimum estimated concentration (roughly equivalent to a "baseline" concentration, but see below) and the slope is the estimate of sensitivity. Further, the slope can be used to make predictions or best estimates of concentrations in an organism for aqueous concentrations greater than might have been measured (see section 8.43: p. 385). Many workers now calulate correlation or regression coefficients for metal accumulation data (Empain, 1977; Brooks & Crooks, 1980; Welsh & Denny, 1980; Harding & Whitton, 1981; Lee et al., 1981; Say et al., 1981; Whitton et al., 1982) and slope and intercept values are usually provided with solutions produced by computer (e.g. Fox & Guire, 1976).

Two precautions must be considered, whether enrichment ratios or slopes are to be used. Firstly, several studies (e.g. Brooks & Crooks, 1980; Say et al., 1981; Foster, 1982; Whitton et al., 1982; and the present study) use $\log_{10}$ transformed data in their analysis. The statistical reason for doing so lies in the requirements for a normal distribution in at least the independent variable (Elliot, 1977), although several authors justify this simply to produce a greater correlation. Nonetheless, a straight line is certainly more robust for prediction and will be more meaningful in an applied sense. However, slopes calculated from scalar (untransformed) data will not necessarily be the same as for $\log_{10}$ transformed data. The true y-intercept (i.e. concentration in an organism at theoretical zero concentration in water), if desired for baseline information, must be calculated from scalar data.

8.43 Predictions

Although several factors have been shown (sections 4.62, 5.2, 5.3, 7.2, 7.3) to influence metal accumulation in aquatic bryophytes, the results for Rhynchostegium indicate that the regression of aqueous v. accumulated metals ($\log_{10}$ transformed) for zinc, cadmium and lead fall along fairly straight lines. As shown by multiple regression analysis and experimental studies, deviations away from the line are at least in part a result of environmetal factors. Some variation must also be due to the fluctuating nature of rivers, particularly with respect to flow conditions, which are known to affect concentrations of dissolved substances (Hynes, 1970; Glover & Johnson, 1974). The fact that metal concentrations in aquatic mosses vary less erratically make it possible to claim average or "integrated" values (Whitehead & Brooks, 1969; Empain, 1976b; Empain et al., 1980; the present study).

Reasonable predictions of metal concentrations in water or moss can be made from a calculated regression line, so long as at least the independent variable, x, (aqueous metal) is normally distributed and the resultant regression coefficient (r^2) is great enough to assume with an acceptable degree of certainty (e.g. 95%: $p < 0.05$) that the function follows a straight line (Snedecor & Cochrane, 1967). A significant departure from linearity would require nonlinear estimates to be used. Therefore using the equation for the slope of any x/y array:

$$y = mx + b;$$

where m = slope and b = y-intercept, a prediction of a metal concentration in moss (y) can be made for any hypothetical concentration in water (x). As the confidence band for a particular regression may be nonlinear, the 95% confidence probability of this estimate (Y) can be calculated from $\pm$ the standard error of the estimate:

$$S\,Y = S_{xy} \sqrt{(1/n) + (x^2/\sum x^2)}$$

where n = the number of individual samples (water and moss) in the dataset (Snedecor & Cochrane, 1967). Most statistics packages now available (e.g. BMDP: Dixon et al., 1981; MIDAS: Fox & Guire, 1976; SPSS: Nie et al., 1975) calculate solutions for these problems and many of the more powerful hand calculators (e.g. Hewlett-Packard Company, 1974) have relatively simple preprogrammed routines for their solution.

The utility of the regression data for zinc, cadmium and lead accumulation in apical tips and whole plants of Rhynchostegium (section 4.62) is demonstrated using a hypothetical example (Table 8.01). In

Table 8.01. Comparison of predicted concentrations of zinc, cadmium and lead in Rhynchostegium, based on a hypothetical 0.1 mg l^{-1} aqueous concentration of each respective element (based on regression of log [moss]: log [water], section 4.62; moss concentrations in $\mu g\ g^{-1}$).

element	fraction	equation	predicted moss concentration	95% confidence interval
Zn	tips	$y = 0.597x + 3.75$	1430	1370 - 1510
	whole	$y = 0.479x + 3.96$	2900	2730 - 3080
Cd	tips	$y = 0.759x + 3.40$	440	270 - 710
	whole	$y = 0.494x + 2.87$	240	140 - 420
Pb	tips	$y = 0.934x + 4.54$	4040	3020 - 5400
	whole	$y = 0.963x + 4.98$	10300	7660 - 14000

each case a predicted concentration in the moss has been accompanied by a range within which 95% of all observations can be expected to be found. Such an exercise also illustrates that differences in both the slope and relative error exist between metals and plants fractions. Further, in not all cases did the plant fraction with the greatest enrichment at the hypothetical concentration have the greatest sensitivity (estimated by the slope). It should be clear that this model is not only more useful than enrichment ratios as a predictive tool, but also more realistic.

8.44 Methodology

Although much of the effort of this study was not directed towards testing specific methods, data from several sections have proven to be of some relevance to the evaluation of aquatic bryophytes as monitors.

Fundamental to any specific approach are the needs of the researcher or water management body. It has been suggested (Whitton et al., 1981b) that whole plants of moss are best used ".. for preliminary studies and extensive surveys .," especially for practical reasons. The reduced time for processing (Wehr et al., in press) is one advantage. Results of the present study show that concentrations of most metals are significantly higher in whole moss (Table 4.12: p. 152), but rates of uptake are likely to be slower (section 7.333: pp. 355-357) and thus less useful in the event of unpredictable release of metals. Based on the present results, use of whole plants should be recommended in cases where routine monitoring of a broad range of rivers is being considered. Transplants (section 7.21: pp. 307-310) and experiments on exchangeability (section 7.332: 354-355) indicate that apical tips of Rhynchostegium are more responsive to rapid changes in concentrations of metals, but will nonetheless retain metals for a considerable period. Results for the alga Lemanea (Harding & Whitton, 1981) support this. Applications of these findings to specific problems are illustrated below in outlines of three possible situations where monitors would be applied.

8.441 Situation 1: routine monitoring

Regular sampling of a large number of river sites is one function of regional water authorities (Hargreaves, 1981). In this situation a simple but reliable method would be desired. The species sampled would depend which sites are normally visited, although comparisons would be simplified by the use of a plant that is found in the greatest variety of rivers and in a broad range of heavy metals. Present results suggest Rhynchostegium would be preferable in this respect. Fontinalis antipyretica may be preferred for ease of recognition in circumstances where sampling is not carried out by a biologist. With either choice, the perennial habit of most aquatic bryophytes (chapter 5) would allow routine sampling year round.

Present results indicate that whole plants would be advantageous because of their significantly greater accumulation (pp. 151-152; 165-167) and simpler laboratory treatment (Wehr et al., in press). A broad picture of heavy metal concentrations would be provided; regression data have shown (pp. 167-191) significant relations for the accumulation of Zn, Cd, Ba and Pb in whole plants. A (dry) weight of roughly 250 mg of whole moss has been shown to be sufficient to detect trace amounts of metals such as Cr and Cd, even in plants from uncontaminated sites. This would be useful for establishing "baseline" concentrations on a regional or country-wide basis, from which water quality criteria could be established. Once collected, dried moss samples could be stored cheaply and without further treatment (Whitton *et al.*, 1981) until large batches of samples are obtained which could be processed more efficiently (e.g. automated analyses), perhaps in a centralized laboratory.

8.442 Situation 2: unpredictable events

Detection of unexpected increases in heavy metals is difficult if only water samples are collected and may also be missed by routine sampling of specific sites. A typical scenario might be a fish kill in a stream within an industrialized region caused by a short-term release of high concentrations of Cd. Bryophytes could be sampled from sites downstream of successive factory effluents to trace the probable source of the discharge after the event.

Uptake and loss experiments (pp. 307-310; 338-340; 353-355) have indicated that metals will be accumulated and retained for several days, even if the uptake period is brief. In these cases, sampling of apical tips would be preferred, as regression results indicate (section 4.62) greater accuracy and experiments indicate (e.g. section 7.21: p. 307-310) a high degree of responsiveness is obtained. Transplants of *Lemanea* support this latter finding (Harding & Whitton, 1981). In a slightly different example, the unexpected increase in zinc pollution in the River Team (pp. 253-260) was clearly detected using aquatic mosses, despite extreme variation in other physicochemical conditions.

8.443 Situation 3: environments without bryophytes

Some sections of rivers may not only lack aquatic bryophytes, but other organisms as well, due to floods, inadequate substrates, or human disturbance. Other water bodies may be inaccessible for practical sampling. In these situations, it is recommended that bryophytes are used as monitors via transplants. Mosses can be transported to specific sites attached to boulders (section 7.2) or placed in perforated bags (e.g. Benson-Evans & Williams, 1976; Prigg, 1977).

This procedure was found to be a simple means of obtaining answers to a variety of questions. In all experiments uptake was rapid. The choice of species and plant fraction used would depend on the question being asked: transplants could be applied to routine or a specific use. If statistical comparisons are to be made, replicates would be desired; present results (e.g. section 7.26: pp. 320-323) have indicated sample variability was usually 10% or less.

8.5 Concluding remarks

8.51 Ecology of aquatic bryophytes

Two interesting problems have come to light regarding the ecology of aquatic bryophytes. The first concerns factors which affect the distribution and abundance of aquatic bryophytes in freshwaters. There seem to be few freshwater environments where these plants are not found. Rhynchostegium riparioides has been found to be particularly ubiquitous. Because it has been reported in lakes (e.g. Light & Lewis Smith, 1976; present study) as well as streams, it seems surprising that it is most commonly reported from rapidly flowing waters (Whitton et al., 1981b) as compared with with deeper, slowly flowing rivers. This may be from a limitation of solid substrate rather than lack of sufficient current. Holmes and Whitton (1981) have suggested riverine bryophytes are favoured in sections of the River Tees with at least moderately rapid current velocity, but with a predictable, regulated flow. Present results suggest this species may be limited by low nutrient levels only when heavy metal concentrations are fairly high; other species appear more restricted. Useful information could be gained through transplants of mosses into environments not previously occupied by particular species. The causes for the apparent historical decline of some species (e.g. Fontinalis squamosa: see section 1.422) could be studied effectively using this approach.

The morphological variability in Rhynchostegium, although found to be considerable, is not uncommon among aquatic bryophytes (Warnstorf et al., 1914; Watson, 1968, Crum & Anderson, 1981). It is surprising, therefore, that although many biometric and experimental studies into variation have been made on bryophytes (Longton, 1981; Schofield, 1981), few are on aquatic species. Morphological variation in Rhynchostegium was related to environmental conditions, which suggests a lack of genetically distinct subspecific taxa. Proof of this, however, will require experiment.

8.52 Metal accumulation and monitoring

It is a relatively simple concept, that organisms are capable of accumulating metals from water, which has lead to a great variety of detailed studies. The literature on this subject covers a wide array of species and is both fundamental and applied in nature. Aquatic bryophytes have proven to be particularly useful experimental organisms in elucidating the mechanisms of metal accumulation. In being sessile (unlike most animals), long lived (unlike most algae) and having relatively simple morphology without true roots (unlike most angiosperms), they represent the ideal experimental organism, when considering conditions of water chemistry at a particular stretch of river. These qualities also recommend bryophytes as monitors.

Starting with the simple relationship of comparing metals in streamwater and metals in moss, it has been possible to build up models which consider the importance of water chemistry variables, the type of plant fraction and growth form on accumulation. Many of these correlative relationships were tested experimentally. Significant negative effects of aqueous magnesium, calcium, manganese and humates were found, while phosphate, nitrate and silica had no apparent effect. The importance of differences in tolerance must also be recognized. Results have suggested differences in the rate and possibly the mechanism of uptake in different elements as well. It would be of interest to examine whether these suggestions are supported by electon microscope or autoradiograph studies on subcellular localization. One profitable line would be to compare such localization after different periods of exposure. If the widely observed slow loss of metals is affected by binding, differences would appear with increasing periods of accumulation or between different populations. Also of interest is the chemical nature of interference by calcium or humic acids. Inhibition in the former follows an inverse log relation, while in the latter the effect is linear. Perhaps competitive relations follow for one relation specific binding by the other.

The "simple" bivariate model of accumulation is nonetheless a very robust one. Many factors alter what might be a more close relationship between metals in moss and metals in water, but the regression analysis shows that it functions well as a predictive tool. Mead (1971) explains that although inelegant, many of the most straightforward models (e.g. temperature & volume effects on the pressure of a gas) are the most practical and demonstrative.

SUMMARY

1. An integrated field and laboratory study was made of the ecology of aquatic bryophytes and their accumulation of heavy metals in rivers in northern England. Results were used to evaluate the effectiveness of bryophytes as monitors of heavy metal pollution.

2. An intensive survey of 105 sites, representing a total of 71 different streams, rivers and other lotic habitats, was carried out. Samples of *Rhynchostegium riparioides* and water were collected for chemical analysis in six-week period. A one year, monthly survey of seven sites was conducted along similar lines to the intensive survey; *Amblystegium riparium* and *Fontinalis antipyretica* were each sampled from one site and *Rhynchostegium* from all seven.

3. Ecological results of the two surveys indicated that *Rhynchostegium* occurred in a great diversity of environments, including springs, small upland streams, mine adits and hushes, large lowland rivers and even a sewage effluent. The broad range of chemical variables are indicative of its ecological ubiquity:

		min	max
pH		6.6	9.8
filtrable reactive PO -P	(μg l^{-1})	< 1.5	6000
NO_3-N	(μg l^{-1})	7.5	31900
Cl^-	(mg l^{-1})	5.0	355
Ca (filtrable)	(mg l^{-1})	3.7	108
Zn (filtrable)	(mg l^{-1})	< 0.006	6.9

Results indicated that estimates of "ecological limits" of an aquatic bryophyte, if based solely on one time of year, may be inaccurate.

4. Multiple regression indicated that the principal factor significantly related to accumulation of Zn, Cd, Ba and Pb by *Rhynchostegium* was the concentration of the respective metal in water. This was true for both apical tips and whole plants. The analysis indicated that chemical variables had a significant (+ or -) effect on metal uptake. For example, accumulation of Zn and Ba by tips was negatively correlated with aqueous calcium. The most common positive factor was Mn in the moss. Mn was significantly correlated with accumulation of Co, Ni, Zn and Ba in tips and whole plants, while Fe in the moss significantly correlated with accumulation of Cu and Pb in both these plant fractions.

5. Pairwise t-tests indicated that whole plants had significantly greater concentrations of Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Cd, Ba and Pb than than tips; only Na and K were greater in tips. A time-series analysis of seasonal accumulation data suggested that these results satisfy the predictions of multiple regression and that there was little evidence of seasonal changes in the mosses. On average, greater concentrations of metals were measured in field populations of *Rhynchostegium* than in *Amblystegium riparium* (20%) and *Fontinalis antipyretica* (four times).

6. A biometric study of morphological variation in *Rhynchostegium* was carried out on 105 populations collected during the intensive survey. Marked differences in gametophytic characters between populations were recorded and classified using cluster analysis. Several highly variable characters identified from the classification were significantly correlated with water chemistry variables indicative of organic pollution. No evidence was found for these differences in morphology affecting metal accumulation.

7. Experiments were conducted in the field (using transplants) to examine factors affecting metal uptake *in situ*. Cross-transplants above and below a zinc effluent demonstrated that uptake proceeded much more rapidly than did loss. Interpopulation differences in Zn uptake were found between five different populations of *Rhynchostegium*, all from low metal streams. A comparison of "metal-tolerant" and "non-tolerant" populations showed no significant difference in Zn uptake, but uptake of Pb was nearly three times greater in the "metal-tolerant" moss, when both were transplanted to a common stream.

8. Laboratory studies found that EDTA, humic acids, Mg, Ca and Mn significantly reduced uptake of ^{65}Zn, while pH, PO_4-P, NO_3-N and Si had no significant effect. Fractionation studies showed that greater Zn and Pb concentrations were associated with Mn- and Fe-rich coatings on the mosses, thus confirming results of multiple regression. However, rates of ^{65}Zn uptake by fractions with greater amounts of Mn and Fe, as was was common in whole mosses, were considerably slower.

9. It is suggested that aquatic bryophytes provide a reliable, "moving-average" of aqueous metals in streams and rivers. Given a concentration of either an aqueous or accumulated metal, a prediction could be made of the concentration in the other component, within 95% confidence limits. Experiments substantiated field data that apical tips of moss were more responsive to changes in aqueous metals than whole plants, and the applications of these differences for monitoring are discussed. Specific guidelines are given for the use of aquatic bryophytes, particularly *Rhynchostegium riparioides*, in monitoring heavy metal pollution in rivers.

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Appendix 1. Physicochemical variables from intensive survey of 105 Rhynchostegium sites. Reaches are listed in numerical order (temperature in ^{o}C; conductivity in $\mu S\ cm^{-1}$; alkalinity in meq l^{-1}; nitrogen and phosphorus in $\mu g\ l^{-1}$; other anions in mg l^{-1}.

stream -reach	temp	O.D. 240nm	O.D. 254nm	O.D. 420nm	cond	pH	total alk	NH4-N	NO2-N	NO3-N	FRP-P	FOP-P	F	Si	SO4-S	Cl	%sat O2
0005-43	13.8	0.057	0.042	0.001	784	8.6	7.12	17.4	15.2	1570	138	3.0	0.168	3.95	72.0	32.0	106
0008-07	8.6	0.194	0.165	0.016	98	7.9	1.10	25.8	3.2	222	7.8	17.7	0.430	1.00	4.8	12.8	103
0008-09	9.0	0.197	0.170	0.016	200	8.2	1.26	34.9	2.9	214	6.4	11.1	0.580	1.25	6.3	12.1	100
0008-17	14.6	0.117	0.097	0.006	730	8.5	1.76	6.8	6.2	273	3.6	8.9	0.810	1.55	13.1	155.0	104
0008-40	13.5	0.240	0.204	0.016	290	8.5	1.42	9.4	6.8	455	12.6	13.5	0.502	0.80	9.4	75.0	110
0008-65	12.8	0.101	0.071	0.004	688	7.9	4.14	161	71.2	3500	242	129	0.360	1.00	72.0	78.0	105
0009-50	16.2	0.411	0.356	0.037	145	8.2	0.86	14.2	4.3	192	16.7	8.8	0.148	0.95	3.5	9.1	104
0009-65	16.8	0.318	0.271	0.027	260	8.7	1.54	20.7	5.6	788	29.2	12.9	0.180	1.25	6.4	16.3	119
0009-80	17.0	0.346	0.262	0.023	410	8.4	2.08	91.2	72.0	4960	439	54.0	0.165	1.25	17.7	25.5	116
0012-45	12.9	0.125	0.107	0.013	305	8.2	1.46	10.2	2.6	135	2.3	5.2	1.03	2.25	16.3	12.1	110
0014-14	12.1	0.111	0.080	0.007	265	8.6	1.29	23.7	6.4	2980	9.1	14.1	0.088	3.80	19.8	29.5	110
0015-15	13.5	0.370	0.326	0.030	96	7.7	0.48	7.9	2.4	39.3	3.2	8.8	0.075	2.00	3.2	8.2	102
0015-18	13.0	0.333	0.293	0.029	115	7.6	0.54	13.5	2.0	44.5	3.5	5.7	0.121	1.80	3.2	8.4	100
0015-98	11.6	0.213	0.182	0.016	170	7.7	1.02	15.1	1.9	188	4.1	4.8	0.235	2.10	4.6	10.4	103
0024-03	10.0	0.084	0.066	0.011	610	7.0	3.32	< 5.0	2.4	1020	< 1.5	4.6	0.135	5.80	73.0	42.0	95
0024-05	11.1	0.099	0.071	0.003	585	7.7	2.13	16.2	14.6	2230	5.0	7.4	0.171	6.50	73.0	52.0	98
0024-20	13.6	0.315	0.190	0.014	780	7.7	3.31	1990	132	11900	828	700	0.343	7.65	70.0	61.0	88
0024-40	14.1	0.235	0.153	0.010	690	8.0	2.91	548	185	8560	526	252	0.275	6.15	80.0	45.0	93
0024-56	15.0	0.253	0.163	0.012	640	8.1	2.72	132	195	10500	465	146	0.291	6.85	55.0	48.0	104
0048-90	12.5	0.175	0.145	0.017	295	8.1	1.14	16.0	2.5	150	17.0	8.1	0.259	1.65	14.8	9.7	110
0048-98	11.9	0.163	0.136	0.019	315	8.0	1.20	14.2	1.5	249	5.2	14.3	0.239	1.45	16.3	9.6	116
0055-30	11.7	0.264	0.225	0.022	195	8.0	1.11	11.4	2.2	133	4.1	< 3.0	0.180	0.75	4.7	7.7	113
0055-31	11.0	0.256	0.219	0.025	220	7.9	1.07	11.6	1.0	144	2.9	7.4	0.195	1.10	4.8	7.8	114
0061-05	12.0	0.236	0.206	0.019	174	7.7	1.00	23.2	1.5	133	3.0	4.8	0.210	1.25	4.7	11.7	103
0061-08	12.5	0.168	0.144	0.015	250	8.0	1.39	171	2.0	440	5.9	6.2	0.66	2.20	8.3	11.3	100
0061-50	14.2	0.184	0.154	0.013	185	7.9	0.96	46.5	4.2	534	13.2	10.7	0.305	1.85	6.1	19.5	107
0081-30	9.3	0.165	0.141	0.012	217	8.0	1.47	19.8	1.4	52.1	2.0	6.6	0.75	1.55	18.7	12.6	100
0081-50	10.0	0.176	0.151	0.011	185	8.3	1.42	13.9	1.0	119	3.2	3.0	0.475	1.55	12.0	11.9	108
0081-95	12.2	0.199	0.170	0.016	195	8.5	1.48	12.8	1.8	206	16.0	11.5	0.430	1.65	8.2	14.1	100
0085-50	15.5	0.134	0.114	0.021	228	8.3	1.77	11.9	1.0	109	< 1.5	5.0	0.362	2.00	13.6	10.5	100
0085-85	14.2	0.160	0.136	0.021	212	8.3	1.77	9.5	1.0	88.6	2.0	6.2	0.258	1.85	8.6	16.0	103
0090-95	13.0	0.102	0.084	0.004	184	7.8	1.07	67.4	1.2	339	21.6	17.3	0.252	3.35	4.5	11.0	97
0101-05	9.4	0.195	0.149	0.011	370	8.0	2.04	39.5	< 1.0	424	< 1.5	10.5	0.288	2.20	17.1	9.8	102
0102-85	7.8	0.234	0.196	0.024	165	7.3	0.77	12.1	< 1.0	116	6.5	5.5	0.230	1.05	7.2	7.2	106
0123-50	10.6	0.141	0.120	0.004	165	7.7	0.77	10.5	1.0	313	3.3	5.6	0.102	1.80	5.1	14.9	111

Appendix 1, continued.

stream -reach	temp	O.D. 240nm	O.D. 254nm	O.D. 420nm	cond	pH	total alk	NH4-N	NO2-N	NO3-N	FRP-P	FOP-P	F	Si	SO4-S	Cl	%sat O2
0166-15	10.2	0.615	0.527	0.063	120	7.3	0.69	12.6	3.7	102	8.7	7.7	0.055	1.60	4.0	7.4	100
0166-30	12.2	0.575	0.496	0.060	162	7.6	1.01	13.9	3.5	222	13.1	5.8	0.158	1.65	3.7	7.9	108
0166-40	13.2	0.530	0.456	0.054	192	7.9	1.23	15.3	3.2	457	11.7	8.6	0.123	1.90	4.3	7.7	102
0167-50	11.0	0.152	0.124	0.010	332	7.9	2.53	20.7	< 1.0	250	< 1.5	13.6	0.118	1.50	13.4	7.8	103
0196-35	10.4	1.101	0.958	0.126	102	7.2	0.53	17.7	4.0	114	19.1	4.8	0.056	1.50	3.7	8.8	103
0196-50	11.0	0.925	0.805	0.105	133	7.3	0.72	13.9	4.0	89.8	13.7	9.4	0.075	1.96	3.7	8.5	100
0196-60	13.0	0.725	0.633	0.086	145	7.2	0.94	11.4	3.4	132	11.6	4.0	0.166	1.68	4.9	9.6	106
0196-90	10.6	0.665	0.579	0.076	155	7.6	1.14	16,0	2.8	224	9.6	12.3	0.190	2.00	4.8	8.2	103
0197-50	8.3	0.438	0.377	0.048	162	7.6	0.94	13.9	3.4	407	10.9	10.5	0.058	2.35	4.9	8.3	111
0197-90	10.4	0.283	0.242	0.033	210	7.9	1.44	11.2	1.4	473	3.8	10.5	0.056	2.45	4.7	9.0	98
0198-95	10.5	0.536	0.467	0.063	153	7.6	0.91	9.8	3.1	268	9.8	5.0	0.62	1.95	4.6	7.3	101
0255-10	13.5	0.196	0.163	0.023	107	6.9	0.10	194	4.4	1350	46.7	8.3	0.046	2.50	5.8	13.8	100
0255-20	13.8	0.161	0.132	0.019	150	7.1	0.28	392	18.3	1710	72.5	30.5	0.048	3.60	7.0	20.9	101
0255-25	14.0	0.150	0.118	0.016	205	7.1	0.56	316	23.0	1360	173	66.0	0.066	3.65	10.4	26.5	94
0255-80	15.2	0.191	0.146	0.018	368	7.2	0.57	1570	135	3230	521	271	0.064	3.35	30.0	26.2	98
0258-95	13.9	0.134	0.102	0.012	205	7.6	0.60	394	27.9	1020	81.3	72.7	0.072	4.25	11.3	26.2	104
0259-50	13.5	0.247	0.111	0.011	360	7.6	0.91	1760	< 1.0	13700	26.4	22.2	0.078	3.56	19.3	20.9	96
0261-20	14.0	0.176	0.143	0.016	245	7.4	0.58	41.4	7.3	1340	21.0	20.8	0.074	3.55	8.5	36.8	99
0262-60	13.1	0.365	0.269	0.032	420	8.2	2.90	66.4	15.9	7810	71.6	57.4	0.064	1.05	6.4	14.3	99
0262-65	13.0	0.323	0.250	0.032	380	8.1	2.41	166	38.1	5060	128	104	0.072	2.35	8.4	17.0	96
0265-10	14.0	0.105	0.081	0.012	138	7.1	0.32	96.4	8.1	1810	14.6	12.9	0.053	3.60	8.5	40.5	99
0265-20	14.0	0.101	0.072	0.010	252	7.3	0.45	152	25.8	2590	23.7	< 3.0	0.061	3.25	10.5	39.2	98
0265-30	14.2	0.091	0.063	0.011	510	7.4	0.71	338	40.3	1650	15.5	16.3	0.088	4.05	3.2	54.0	99
0274-80	13.5	0.110	0.091	0.010	254	7.2	0.47	82.9	7.8	443	8.0	8.1	0.056	5.50	9.4	38.5	97
0275-95	12.5	0.092	0.072	0.008	440	8.2	3.31	27.6	9.8	602	19.0	28.8	0.084	3.70	10.0	17.0	100
0276-90	9.8	0.763	0.665	0.090	134	7.4	0.75	11.2	3.1	158	8.4	11.5	0.130	1.75	4.0	7.5	101
0277-97	10.5	0.845	0.720	0.069	173	7.8	1.28	11.2	4.8	78.5	9.1	7.0	0.412	1.60	3.8	8.8	99
0278-75	12.4	0.037	0.026	0.000	327	8.1	2.14	20.7	< 1.0	606	< 1.5	9.2	1.20	3.15	11.4	11.8	100
0279-98	10.5	0.020	0.014	0.000	333	8.3	2.60	8.9	1.0	550	3.0	5.9	0.67	3.30	7.8	11.1	99
0281-95	13.2	0.322	0.268	0.022	380	8.1	2.52	15.7	3.0	352	16.0	12.2	0.097	3.25	10.0	19.5	98
0282-75	11.5	0.496	0.427	0.057	244	7.9	1.64	15.8	3.3	597	9.3	5.1	0.082	2.55	6.0	9.6	100
0283-95	12.4	0.392	0.341	0.043	173	7.6	1.04	11.6	1.6	267	7.0	6.9	0.082	1.92	4.4	8.6	100
0284-85	12.3	0.167	0.149	0.007	124	7.6	0.63	10.2	1.6	132	2.9	5.5	0.075	2.50	4.3	10.7	113
0285-65	9.0	0.158	0.127	0.012	206	7.7	1.08	10.0	< 1.0	151	2.1	9.7	0.077	1.40	8.0	6.9	110
0286-05	13.8	0.182	0.157	0.017	193	8.6	1.56	11.2	1.0	109	7.6	10.2	0.285	1.75	8.4	15.1	110

Appendix 1, continued.

stream -reach	temp	O.D. 240nm	O.D. 254nm	O.D. 420nm	cond	pH	total alk	NH4-N	NO2-N	NO3-N	FRP-P	FOP-P	F	Si	SO4-S	Cl	%sat O2
0287-85	6.5	0.278	0.227	0.028	124	7.5	0.72	9.3	1.0	141	3.0	< 3.0	0.100	1.05	4.1	6.4	103
0288-98	9.5	0.125	0.100	0.009	300	7.8	1.66	35.3	1.1	791	4.4	6.3	0.077	1.85	10.7	18.2	106
0289-98	10.0	0.150	0.129	0.016	243	7.4	1.84	9.5	< 1.0	235	1.8	5.0	0.083	1.55	4.5	9.4	93
0290-95	10.5	0.061	0.050	0.011	52	7.0	0.20	6.7	< 1.0	242	3.0	5.2	0.030	0.96	1.7	7.1	99
0291-50	11.7	0.056	0.046	0.012	56	6.8	0.14	7.9	< 1.0	338	< 1.5	6.4	0.034	0.94	3.5	7.0	100
0292-98	10.7	0.113	0.096	0.015	50	6.8	0.16	26.7	< 1.0	167	7.4	8.3	0.025	1.02	1.7	5.8	97
0293-99	12.3	0.367	0.292	0.033	77	7.0	0.32	16.5	1.5	209	12.3	15.2	0.048	1.32	2.9	7.9	101
0296-90	11.2	0.071	0.061	0.009	44	7.1	0.15	5.6	< 1.0	159	< 1.5	6.4	0.032	0.75	1.3	5.2	103
0300-40	10.8	0.275	0.234	0.027	48	6.8	0.16	7.4	< 1.0	29.9	1.5	8.1	0.033	0.64	1.5	5.8	101
0300-45	10.5	0.264	0.223	0.023	78	7.0	0.32	24.2	1.0	71.2	3.0	4.1	0.038	0.84	1.9	9.8	103
0303-90	9.0	0.296	0.249	0.021	229	7.9	1.72	8.1	1.5	201	5.5	9.1	0.078	2.50	4.7	9.2	98
0304-95	10.6	0.294	0.252	0.033	160	7.6	0.98	11.6	1.6	261	5.3	6.8	0.275	1.95	4.3	7.9	103
0305-01	10.6	0.000	0.000	0.000	221	7.4	1.36	6.3	< 1.0	129	< 1.5	4.6	0.435	2.25	6.7	14.0	110
0306-95	12.8	0.165	0.142	0.019	180	8.1	1.39	8.6	< 1.0	63.2	2.0	34.3	0.369	1.75	3.9	7.4	100
0307-98	11.0	0.132	0.113	0.008	270	8.2	2.19	6.4	< 1.0	146	< 1.5	5.6	1.07	2.40	7.1	8.4	102
0309-95	10.0	0.066	0.059	0.004	326	8.2	2.46	17.4	< 1.0	174	< 1.5	6.0	1.02	2.10	11.3	10.6	101
0310-90	12.0	0.076	0.063	0.003	177	7.8	0.90	24.2	< 1.0	138	1.8	6.8	0.155	2.90	3.7	9.7	110
0312-90	11.8	0.081	0.063	0.011	300	8.1	2.99	9.4	< 1.0	438	9.7	7.1	0.142	3.80	10.1	13.5	108
0312-95	11.6	0.146	0.123	0.017	192	8.0	2.01	9.5	< 1.0	226	2.4	8.3	0.088	1.70	5.0	9.1	109
0314-98	8.7	0.449	0.386	0.051	231	7.9	1.62	15.6	2.6	335	7.8	7.6	0.064	1.55	2.9	8.0	101
0315-98	10.4	0.066	0.054	0.004	210	7.8	1.38	11.4	1.8	111	3.3	< 3.0	0.067	2.05	4.4	11.0	99
0316-90	9.5	0.179	0.151	0.020	308	8.1	2.12	40.5	1.4	489	20.0	11.8	0.048	2.65	5.9	11.3	100
0317-50	12.5	0.240	0.230	0.021	265	8.3	1.80	11.6	1.0	388	7.0	11.9	0.180	2.15	5.5	11.2	101
0318-95	11.4	0.063	0.050	0.004	312	7.8	2.07	23.0	1.4	487	8.7	5.9	1.30	3.35	8.2	11.2	99
0319-10	7.5	0.000	0.000	0.000	420	7.1	2.60	15.3	< 1.0	652	< 1.5	5.4	1.05	4.65	11.9	12.9	61
0320-95	13.5	0.332	0.284	0.026	269	8.2	1.40	7.9	< 1.0	160	1.7	3.7	0.053	2.65	10.1	13.0	105
0321-95	10.8	0.063	0.053	0.002	130	7.4	0.60	10.5	< 1.0	118	3.0	9.3	0.400	2.65	4.1	10.7	101
0354-98	15.0	0.697	0.596	0.061	227	7.9	1.28	26.3	5.4	79.0	15.6	18.7	0.093	1.40	4.7	9.2	100
0355-95	13.8	0.473	0.397	0.033	245	8.0	1.93	9.4	3.1	39.3	7.3	17.7	0.106	1.70	4.2	8.6	106
0356-85	10.5	0.061	0.045	0.001	372	8.2	2.68	21.2	1.1	613	8.8	14.0	0.200	2.65	7.0	12.3	101
0357-40	16.6	0.564	0.476	0.032	226	7.8	1.34	16.5	2.9	9.9	34.5	15.5	0.139	1.95	6.4	6.7	100
0357-45	17.5	0.480	0.406	0.026	250	8.0	1.40	13.5	2.5	7.5	33.4	14.8	0.135	1.80	6.6	7.4	106
0358-90	16.5	0.585	0.501	0.048	120	7.7	0.76	14.9	3.7	49.5	5.2	< 3.0	0.310	1.15	3.1	5.7	102
0359-90	15.5	0.208	0.180	0.016	236	8.0	1.28	13.2	1.0	102	2.7	5.9	0.73	2.80	7.8	6.9	98
0360-01	13.0	0.102	0.074	0.008	510	7.1	0.50	107	21.0	31900	3180	294	1.02	9.90	58.0	77.0	82

Appendix 2. Total and filtrable metals in waters from intensive survey of 105 Rhynchostegium sites, part 1: Na through Fe (concentrations in mg l^{-1}; detection limit of total Cr 10 times greater via concentration, see section 4.22).

stream -reach	Na		Mg		K		Ca		Cr		Mn		Fe	
	T	F	T	F	T	F	T	F	T	F	T	F	T	F
0005-43	60.0	59.6	61.6	60.0	15.2	14.8	90.4	88.0	0.003	<0.01	0.088	0.088	0.22	0.17
0008-07	6.2	6.4	1.68	1.68	1.20	1.20	24.8	24.8	0.002	<0.01	0.022	0.020	1.15	0.10
0008-09	6.2	5.8	3.84	3.80	1.52	1.48	29.0	29.0	0.001	<0.01	0.024	0.024	0.72	0.10
0008-17	80.2	78.4	6.64	6.60	6.72	5.64	86.0	86.0	0.002	<0.01	0.144	0.144	0.17	0.06
0008-40	28.0	28.0	4.04	4.16	3.36	3.24	43.2	43.6	0.003	<0.01	0.036	0.032	0.31	0.08
0008-65	89.0	87.8	32.4	31.2	10.0	9.60	80.4	78.0	0.003	<0.01	0.128	0.112	0.25	0.15
0009-50	5.8	5.6	2.20	2.24	0.96	0.92	18.3	18.6	<0.001	<0.01	0.012	0.008	0.28	0.18
0009-65	8.0	8.2	4.88	4.80	1.56	1.52	31.2	30.9	<0.001	<0.01	0.016	0.016	0.19	0.10
0009-80	16.4	16.0	10.8	10.4	3.20	3.08	46.4	45.6	0.003	<0.01	0.030	0.022	0.17	0.09
0012-45	8.4	7.8	6.04	6.00	2.60	2.44	38.8	38.6	<0.001	<0.01	0.216	0.216	0.12	0.06
0014-14	17.2	17.2	13.6	13.6	3.00	2.88	27.9	28.0	0.001	<0.01	0.014	0.014	0.12	0.12
0015-15	5.6	4.8	1.84	1.84	0.72	0.76	9.84	10.1	<0.001	<0.01	0.018	0.016	0.27	0.24
0015-18	5.2	5.2	2.08	2.04	0.80	0.80	12.4	11.7	<0.001	<0.01	0.032	0.012	0.32	0.12
0015-98	7.4	7.0	3.24	3.20	1.20	1.20	21.6	21.3	<0.001	<0.01	0.020	0.016	0.12	0.06
0024-03	23.6	23.6	48.0	48.0	5.84	5.68	84.4	85.2	0.003	<0.01	0.77	0.75	0.68	0.34
0024-05	16.4	16.8	33.2	32.8	5.76	5.72	88.0	85.6	0.004	<0.01	0.408	0.400	0.32	0.08
0024-20	76.4	75.0	23.6	22.8	11.6	11.2	84.4	80.8	0.006	<0.01	0.392	0.332	0.76	0.25
0024-40	60.0	60.0	23.6	22.8	9.6	9.6	80.8	78.8	0.003	<0.01	0.328	0.304	0.70	0.05
0024-56	56.4	55.8	21.6	20.9	9.6	9.4	76.8	74.2	0.004	<0.01	0.336	0.298	0.88	0.20
0048-90	6.4	6.4	5.00	5.00	1.80	1.72	31.3	31.3	0.002	<0.01	0.044	0.032	0.24	0.14
0048-98	6.6	6.4	5.68	5.64	1.88	1.92	34.5	33.9	0.002	<0.01	0.038	0.036	0.25	0.12
0055-30	4.0	3.6	2.26	2.20	0.84	0.80	23.8	23.8	<0.001	<0.01	0.012	0.010	0.24	0.14
0055-31	4.4	4.4	2.52	2.48	0.96	0.88	24.4	24.0	0.001	<0.01	0.018	0.010	0.18	0.12
0061-05	7.6	7.2	4.56	4.44	2.00	2.00	19.3	18.6	<0.001	<0.01	0.022	0.008	0.25	0.18
0061-08	14.0	13.8	5.40	5.32	3.88	3.64	25.1	24.9	<0.001	<0.01	0.018	0.018	0.19	0.13
0061-50	14.8	14.8	3.64	3.52	2.28	2.16	15.2	14.5	<0.001	<0.01	0.036	0.016	0.25	0.13
0081-30	11.4	10.6	7.48	7.48	2.84	2.80	36.8	36.8	<0.001	<0.01	0.036	0.028	0.14	0.18
0081-50	9.8	9.2	6.40	6.16	2.24	2.12	32.0	30.8	<0.001	<0.01	0.032	0.024	0.20	0.15
0081-95	12.0	12.0	5.60	5.52	2.72	2.56	31.2	30.8	<0.001	<0.01	0.014	0.014	0.18	0.12
0085-50	7.8	7.2	6.32	6.40	2.12	2.12	40.0	41.2	<0.001	<0.01	0.016	0.012	0.06	0.03
0085-85	7.4	7.2	5.20	5.12	1.76	1.76	40.0	38.8	<0.001	<0.01	0.016	0.014	0.13	0.06
0090-95	7.0	6.8	4.32	4.32	2.24	2.20	20.0	20.0	<0.001	<0.01	0.024	0.024	0.17	0.06
0101-05	6.2	6.2	7.40	6.80	1.96	1.88	51.2	46.8	<0.001	<0.01	0.040	0.030	0.13	0.02
0102-85	4.4	4.4	2.72	2.72	1.00	0.96	17.8	17.9	<0.001	<0.01	0.020	0.014	0.20	0.10
0123-50	8.8	8.8	2.92	2.92	1.28	1.24	16.6	16.2	<0.001	<0.01	0.024	0.014	0.09	0.07

Appendix 2, continued.

stream -reach	Na T	Na F	Mg T	Mg F	K T	K F	Ca T	Ca F	Cr T	Cr F	Mn T	Mn F	Fe T	Fe F
0166-15	4.0	4.4	1.96	1.72	0.56	0.56	17.8	16.2	<0.001	<0.01	0.052	0.032	0.56	0.32
0166-30	4.8	4.8	2.28	2.24	0.64	0.64	22.4	22.4	<0.001	<0.01	0.020	0.008	0.38	0.26
0166-40	4.6	5.0	2.84	2.72	0.68	0.68	26.0	25.6	<0.001	<0.01	0.016	0.012	0.29	0.24
0167-50	5.2	5.2	5.92	6.96	0.40	0.40	50.4	56.0	<0.001	<0.01	0.033	0.030	0.72	0.58
0196-35	4.8	4.8	2.28	2.20	0.44	0.44	14.3	14.4	<0.001	<0.01	0.044	0.040	0.04	0.04
0196-50	5.0	4.8	2.88	2.76	0.44	0.44	19.5	18.5	<0.001	<0.01	0.022	0.016	0.64	0.53
0196-60	5.0	4.8	3.40	3.40	0.96	0.96	22.4	22.8	<0.001	<0.01	0.016	0.012	0.48	0.48
0196-90	5.6	5.2	3.36	3.28	1.36	1.36	26.0	25.6	<0.001	<0.01	0.010	0.008	0.44	0.38
0197-50	4.6	4.4	2.40	2.36	2.08	2.00	22.4	22.0	<0.001	<0.01	0.008	0.012	0.20	0.14
0197-90	5.0	5.4	3.48	3.40	3.96	3.72	30.8	29.6	<0.001	<0.01	0.014	0.008	0.12	0.07
0198-95	4.4	4.2	1.88	1.68	1.88	2.00	22.8	20.8	<0.001	<0.01	0.028	0.028	0.34	0.28
0255-10	7.6	7.4	3.00	2.88	1.84	1.80	6.60	6.32	<0.001	<0.01	0.136	0.112	0.58	0.35
0255-20	13.8	13.6	4.28	4.16	1.44	1.40	9.80	9.64	<0.001	<0.01	0.096	0.076	0.30	0.16
0255-25	11.4	10.4	4.68	4.76	2.04	2.00	14.2	14.5	<0.001	<0.01	0.096	0.096	0.52	0.21
0255-80	31.8	31.0	6.60	6.60	3.88	3.72	24.0	24.0	0.013	<0.01	0.200	0.18	0.58	0.27
0258-95	14.4	15.4	6.04	6.00	1.84	1.88	19.0	18.7	0.004	<0.01	0.042	0.037	0.28	0.08
0259-50	38.2	36.4	7.52	6.80	1.80	1.80	24.0	22.0	0.16	0.12	0.064	0.032	0.26	0.04
0261-20	19.0	18.8	4.96	4.76	1.72	1.52	18.4	17.6	0.003	<0.01	0.080	0.056	0.80	0.32
0262-60	16.4	16.4	3.20	3.20	1.44	1.44	65.2	66.2	0.002	<0.01	0.036	0.024	0.31	0.12
0262-65	16.8	16.8	3.56	3.52	2.20	2.08	52.4	52.4	0.003	<0.01	0.072	0.032	0.54	0.20
0265-10	18.4	18.4	4.92	4.88	1.64	1.68	11.9	12.0	0.002	<0.01	0.028	0.022	0.13	0.02
0265-20	19.2	19.4	6.40	6.40	2.20	2.20	15.8	15.6	0.002	<0.01	0.032	0.026	0.12	0.08
0265-30	41.4	41.0	12.0	11.6	3.72	3.60	36.0	35.2	0.004	<0.01	0.424	0.372	0.37	0.06
0274-80	19.4	18.8	6.60	6.60	1.36	1.32	14.4	14.4	<0.001	<0.01	0.168	0.140	0.68	0.16
0275-95	8.2	8.2	4.52	4.52	1.40	1.40	74.4	74.0	<0.001	<0.01	0.056	0.026	0.20	0.07
0276-90	4.4	4.2	2.64	2.64	0.48	0.36	17.0	17.4	<0.001	<0.01	0.020	<0.004	0.34	0.30
0277-97	4.4	4.6	2.72	2.70	0.40	0.40	28.4	28.4	<0.001	<0.01	0.028	0.016	0.60	0.46
0278-75	6.0	5.8	5.40	5.04	1.00	0.94	50.4	48.0	<0.001	<0.01	0.038	0.018	0.19	0.03
0279-98	5.2	6.0	8.40	8.00	0.92	0.96	50.0	49.6	<0.001	<0.01	0.014	0.008	0.05	0.02
0281-95	9.2	9.0	6.64	6.40	1.80	1.72	50.4	51.2	<0.001	<0.01	0.026	0.020	0.28	0.15
0282-75	5.8	6.2	4.80	4.44	0.72	0.68	37.6	34.8	<0.001	<0.01	0.010	0.008	0.31	0.25
0283-95	4.6	4.6	2.16	2.12	0.32	0.32	24.8	24.4	<0.001	<0.01	0.008	0.008	0.10	0.09
0284-85	6.4	6.0	2.68	2.68	0.84	0.80	11.8	11.6	<0.001	<0.01	0.010	0.008	0.21	0.17
0285-65	3.7	3.7	3.04	3.04	0.76	0.80	25.2	25.6	<0.001	<0.01	0.046	0.036	0.15	0.11
0286-05	9.8	9.2	4.80	4.84	1.96	1.92	32.8	32.4	<0.001	<0.01	0.018	0.010	0.12	0.06

Appendix 2, continued.

stream -reach	Na T	Na F	Mg T	Mg F	K T	K F	Ca T	Ca F	Cr T	Cr F	Mn T	Mn F	Fe T	Fe F
0287-85	3.4	3.5	2.00	2.00	0.64	0.48	16.3	16.3	<0.001	<0.01	0.016	0.088	0.21	0.15
0288-98	5.2	4.8	5.44	5.40	1.84	1.84	38.0	38.0	<0.001	<0.01	0.012	0.008	0.20	0.09
0289-98	5.4	5.8	4.28	4.28	1.32	1.32	36.8	36.4	<0.001	<0.01	0.012	0.010	0.07	0.03
0290-95	2.8	3.2	0.92	0.92	0.08	0.08	5.24	5.12	<0.001	<0.01	0.012	<0.004	<0.02	<0.02
0291-50	3.8	3.8	0.76	0.76	0.14	0.14	4.76	4.72	<0.001	<0.01	0.008	0.006	0.11	<0.02
0292-98	3.5	3.0	0.84	0.84	0.20	0.20	4.24	4.28	<0.001	<0.01	0.008	0.008	0.04	0.02
0293-99	4.0	4.6	1.56	1.52	0.48	0.48	6.52	6.52	<0.001	<0.01	0.052	0.048	0.29	0.13
0296-90	2.6	2.6	0.72	0.72	0.12	0.08	3.72	3.72	<0.001	<0.01	<0.004	<0.004	<0.02	<0.02
0300-40	3.0	3.0	0.96	0.96	0.12	0.08	4.44	4.40	<0.001	<0.01	0.008	<0.004	0.08	0.03
0300-45	5.6	5.6	1.20	1.20	0.20	0.24	7.04	6.92	<0.001	<0.01	0.020	0.010	0.08	0.07
0303-90	4.8	4.6	3.28	3.28	0.64	0.60	35.6	35.6	<0.001	<0.01	0.012	0.010	0.20	0.12
0304-95	4.4	4.2	2.12	2.12	0.60	0.48	22.1	22.6	<0.001	<0.01	0.020	0.016	0.13	0.12
0305-01	6.8	6.8	3.72	3.68	1.16	1.16	30.8	29.6	<0.001	<0.01	0.008	<0.004	0.04	<0.02
0306-95	4.2	4.0	3.68	3.56	0.76	0.76	28.4	28.0	<0.001	<0.01	0.010	0.008	0.14	0.09
0307-98	5.0	5.0	5.72	5.80	2.28	2.24	41.2	42.0	<0.001	<0.01	<0.004	<0.004	0.08	0.06
0309-95	6.2	6.4	7.84	7.84	2.40	2.48	48.0	48.0	0.002	<0.01	0.014	0.012	0.02	<0.02
0310-90	5.4	5.2	2.24	2.24	0.76	0.68	19.7	19.4	<0.001	<0.01	0.088	0.012	0.36	0.03
0312-90	8.8	8.6	8.00	8.00	2.60	2.60	59.2	59.2	0.002	<0.01	0.010	0.008	0.16	0.02
0312-95	5.8	5.8	4.60	4.60	1.48	1.40	38.4	37.6	<0.001	<0.01	0.010	0.008	0.06	0.02
0314-98	4.2	4.4	2.20	2.16	0.44	0.40	34.8	34.0	<0.001	<0.01	0.008	<0.004	0.17	0.12
0315-98	5.0	5.2	4.40	4.40	0.56	0.56	28.0	28.0	<0.001	<0.01	0.012	0.008	0.15	0.11
0316-90	5.4	5.6	6.32	6.16	0.72	0.68	44.0	42.8	<0.001	<0.01	0.019	0.014	0.12	0.03
0317-50	5.8	5.8	2.20	2.20	0.48	0.44	42.0	42.0	0.003	<0.01	0.018	0.008	0.16	0.06
0318-95	6.0	6.0	5.40	5.40	0.87	0.84	46.0	45.6	<0.001	<0.01	0.018	0.008	0.20	0.04
0319-10	8.8	8.4	7.20	7.20	2.00	2.04	56.4	56.8	<0.001	<0.01	0.012	0.018	0.02	<0.02
0320-95	8.4	8.0	4.48	4.48	1.76	1.76	33.2	32.4	<0.001	<0.01	0.024	0.016	0.10	0.02
0321-95	6.6	6.6	2.44	2.48	0.84	0.84	13.7	13.7	<0.001	<0.01	0.012	0.008	0.10	0.08
0354-98	6.0	5.8	3.40	3.32	1.16	1.08	27.6	27.6	<0.001	<0.01	0.026	0.010	0.60	0.40
0355-95	3.8	3.8	4.00	3.92	1.00	0.96	39.6	38.4	<0.001	<0.01	0.020	0.008	0.29	0.14
0356-85	4.2	4.4	7.00	6.80	2.16	2.04	52.0	50.0	<0.001	<0.01	0.065	0.005	0.53	0.30
0357-40	3.7	3.7	4.40	4.32	0.48	0.40	31.6	30.4	<0.001	<0.01	0.026	0.008	0.47	0.23
0357-45	3.4	3.8	4.20	4.12	0.56	0.56	30.4	29.2	<0.001	<0.01	0.056	0.018	0.42	0.12
0358-90	3.6	3.2	2.28	2.28	0.60	0.60	15.6	15.6	<0.001	<0.01	0.026	0.020	0.48	0.34
0359-90	5.0	5.0	5.60	5.44	1.52	1.56	27.2	26.8	<0.001	<0.01	0.007	0.005	0.12	0.08
0360-01	48.6	48.2	9.60	9.60	10.4	10.4	52.0	49.6	0.008	<0.01	0.132	0.032	0.98	0.09

Appendix 3. Total and filtrable metals in waters from intensive survey of 105 <u>Rhynchostegium</u> sites, part 2: Co through Pb (concentrations in mg l^{-1}; detection limits 10 times greater for total Co, Ni, and Cu via concentration, see section 4.22).

stream -reach	Co		Ni		Cu		Zn		Cd		Ba		Pb	
	T	F	T	F	T	F	T	F	T	F	T	F	T	F
0005-43	0.009	<0.02	0.028	<0.04	0.0044	<0.006	0.020	0.014	0.00011	0.00040	0.76	0.20	0.0020	0.0145
0008-07	0.009	<0.02	0.011	<0.04	0.0026	<0.006	0.092	0.046	0.00058	0.00026	0.15	0.07	0.0125	0.0035
0008-09	0.008	<0.02	0.011	<0.04	0.0038	<0.006	0.12	0.058	0.00046	0.00023	0.16	0.12	0.0085	0.0025
0008-17	0.015	<0.02	0.023	<0.04	0.0074	<0.006	0.11	0.084	0.00053	0.00053	0.91	0.37	0.0130	0.0030
0008-40	0.007	<0.02	0.016	<0.04	0.0037	<0.006	0.084	0.050	0.00029	0.00028	0.20	0.17	0.0110	0.0060
0008-65	0.014	<0.02	0.025	<0.04	0.0044	<0.006	0.042	0.036	0.00018	0.00015	0.65	0.20	0.0025	0.0015
0009-50	0.006	<0.02	0.006	<0.04	0.0099	<0.006	0.024	0.018	0.00014	0.00014	0.13	0.10	0.0110	0.0080
0009-65	0.005	<0.02	0.013	<0.04	0.0028	<0.006	0.020	0.016	0.00019	0.00010	0.15	0.17	0.0045	0.0040
0009-80	0.009	<0.02	0.033	<0.04	0.0086	<0.006	0.020	0.014	0.00022	0.00018	0.15	0.19	0.0045	0.0025
0012-45	0.009	<0.02	0.017	<0.04	0.0080	0.006	0.40	0.33	0.00104	0.00104	0.25	0.10	0.0150	0.0070
0014-14	0.005	<0.02	0.011	<0.04	0.0023	<0.006	0.012	0.010	0.00009	0.00009	0.13	0.17	0.0025	0.0010
0015-15	0.003	<0.02	0.004	<0.04	0.0022	<0.006	0.022	0.010	0.00014	0.00014	0.05	0.07	0.0040	0.0035
0015-18	0.003	<0.02	0.004	<0.04	0.0019	<0.006	0.022	0.012	0.00027	0.00015	0.07	0.07	0.0705	0.0045
0015-98	0.003	<0.02	0.007	<0.04	0.0028	<0.006	0.042	0.028	0.00020	0.00016	0.15	0.13	0.0170	0.0070
0024-03	0.017	<0.02	0.030	<0.04	0.0060	<0.006	0.038	0.036	0.00030	0.00018	0.58	0.20	0.0045	0.0020
0024-05	0.016	<0.02	0.029	<0.04	0.0046	<0.006	0.052	0.046	0.00021	0.00015	0.65	0.20	0.0065	0.0065
0024-20	0.016	<0.02	0.038	<0.04	0.0147	0.009	0.35	0.30	0.00097	0.00081	0.75	0.20	0.0060	0.0025
0024-40	0.014	<0.02	0.029	<0.04	0.0076	0.006	0.26	0.23	0.00036	0.00015	0.73	0.19	0.0045	0.0045
0024-56	0.016	<0.02	0.031	<0.04	0.0082	0.006	0.20	0.18	0.00030	0.00019	0.21	0.09	0.0085	0.0065
0048-90	0.005	<0.02	0.009	<0.04	0.0026	<0.006	1.75	1.62	0.00259	0.00230	0.29	0.16	0.0225	0.0150
0048-98	0.009	<0.02	0.013	<0.04	0.0024	<0.006	1.50	1.50	0.00230	0.00230	0.36	0.19	0.0205	0.0165
0055-30	0.008	<0.02	0.009	<0.04	0.0040	<0.006	0.056	0.054	0.00032	0.00027	0.11	0.09	0.0130	0.0080
0055-31	0.005	<0.02	0.010	<0.04	0.0024	<0.006	0.184	0.156	0.00064	0.00064	0.15	0.07	0.0160	0.0110
0061-05	0.004	<0.02	0.011	<0.04	0.0014	<0.006	0.016	0.012	0.00009	0.00009	0.07	0.07	0.0025	0.0025
0061-08	0.004	<0.02	0.011	<0.04	0.0022	<0.006	0.148	0.136	0.00073	0.00067	0.18	0.09	0.0185	0.0100
0061-50	0.003	<0.02	0.006	<0.04	0.0022	<0.006	0.024	0.020	0.00021	0.00015	0.16	0.10	0.0060	0.0015
0081-30	0.009	<0.02	0.011	<0.04	0.0028	<0.006	0.164	0.156	0.00046	0.00055	0.31	0.10	0.0225	0.0090
0081-50	0.003	<0.02	0.014	<0.04	0.0018	<0.006	0.156	0.140	0.00058	0.00042	0.25	0.09	0.0090	0.0075
0081-95	0.005	<0.02	0.011	<0.04	0.0024	<0.006	0.064	0.048	0.00027	0.00021	0.12	0.12	0.0080	0.0040
0085-50	0.008	<0.02	0.012	<0.04	0.0027	<0.006	0.64	0.59	0.00212	0.00196	0.58	0.24	0.0045	0.0035
0085-85	0.007	<0.02	0.012	<0.04	0.0024	<0.006	0.28	0.25	0.00247	0.00147	0.45	0.21	0.0025	0.0015
0090-95	0.003	<0.02	0.010	<0.04	0.0045	<0.006	0.014	0.010	0.00016	0.00014	0.07	0.05	0.0100	0.0020
0101-05	0.003	<0.02	0.016	<0.04	0.0028	<0.006	0.75	0.70	0.00159	0.00156	0.52	0.12	0.0745	0.0140
0102-85	0.002	<0.02	0.007	<0.04	0.0028	<0.006	0.41	0.40	0.00100	0.00100	0.16	0.07	0.0410	0.0320
0123-50	0.005	<0.02	0.008	<0.04	0.0016	<0.006	0.010	0.008	0.00019	0.00019	0.19	0.10	0.0015	0.0020

Appendix 3, continued.

stream -reach	Co T	Co F	Ni T	Ni F	Cu T	Cu F	Zn T	Zn F	Cd T	Cd F	Ba T	Ba F	Pb T	Pb F
0166-15	0.005	<0.02	0.017	<0.04	0.0060	<0.006	0.038	0.030	0.00026	0.00026	0.12	0.10	0.0035	0.0025
0166-30	0.005	<0.02	0.008	<0.04	0.0048	<0.006	0.062	0.056	0.00043	0.00042	0.38	0.21	0.0245	0.0175
0166-40	0.007	<0.02	0.016	<0.04	0.0044	<0.006	0.054	0.046	0.00048	0.00032	0.23	0.21	0.0200	0.0160
0167-50	0.008	<0.02	0.020	<0.04	0.011	<0.006	0.34	0.33	0.00115	0.00094	0.55	0.18	0.0170	0.0140
0196-35	0.005	<0.02	0.008	<0.04	0.0054	<0.006	0.020	0.016	0.00022	0.00021	0.12	0.11	0.0080	0.0060
0196-50	0.005	<0.02	0.015	<0.04	0.0048	<0.006	0.028	0.022	0.00033	0.00033	0.21	0.18	0.0125	0.0080
0196-60	0.009	<0.02	0.017	<0.04	0.0034	<0.006	0.050	0.046	0.00038	0.00038	0.21	0.22	0.0155	0.0110
0196-90	0.005	<0.02	0.009	<0.04	0.0069	<0.006	0.062	0.052	0.00059	0.00054	0.38	0.25	0.0150	0.0100
0197-50	0.003	<0.02	0.017	<0.04	0.0101	<0.006	0.30	0.24	0.00103	0.00082	0.26	0.19	0.0445	0.0330
0197-90	0.003	<0.02	0.022	<0.04	0.0078	<0.006	0.22	0.15	0.00069	0.00066	0.38	0.25	0.0755	0.0325
0198-95	0.004	<0.02	0.009	<0.04	0.0095	<0.006	0.194	0.174	0.00121	0.00124	0.79	0.66	0.2000	0.1780
0255-10	0.004	<0.02	0.011	<0.04	0.0020	<0.006	0.018	0.012	0.00042	0.00025	0.06	0.08	0.0045	0.0025
0255-20	0.004	<0.02	0.009	<0.04	0.0034	<0.006	0.52	0.47	0.00042	0.00030	0.10	0.05	0.0040	0.0025
0255-25	0.002	<0.02	0.014	<0.04	0.0042	<0.006	0.214	0.184	0.00033	0.00024	0.08	0.08	0.0033	0.0020
0255-80	0.007	<0.02	0.012	<0.04	0.0052	<0.006	0.188	0.152	0.00045	0.00049	0.13	0.11	0.0025	0.0015
0258-95	0.008	<0.02	0.016	<0.04	0.0072	<0.006	0.016	0.016	0.00056	0.00024	0.08	0.06	0.0030	0.0020
0259-50	0.008	<0.02	0.014	<0.04	0.0100	<0.006	0.014	0.012	0.00142	0.00062	0.20	0.06	0.0455	0.0160
0261-20	0.005	<0.02	0.015	<0.04	0.0095	<0.006	0.016	0.012	0.00101	0.00047	0.17	0.16	0.0025	0.0010
0262-60	0.008	<0.02	0.029	<0.04	0.0074	<0.006	0.018	0.016	0.00012	0.00012	0.21	0.22	0.0025	0.0010
0262-65	0.004	<0.02	0.027	<0.04	0.0125	<0.006	0.032	0.024	0.00149	0.00056	0.14	0.18	0.0055	0.0020
0265-10	0.007	<0.02	0.017	<0.04	0.0076	<0.006	0.008	0.008	0.00016	0.00010	0.07	0.04	0.0010	0.0010
0265-20	0.005	<0.02	0.012	<0.04	0.0064	<0.006	0.010	0.012	0.00034	0.00016	0.05	0.05	0.0060	0.0010
0265-30	0.008	<0.02	0.020	<0.04	0.0070	<0.006	0.012	0.012	0.00010	0.00010	0.30	0.10	0.0020	0.0020
0274-80	0.005	<0.02	0.015	<0.04	0.0076	<0.006	0.020	0.018	0.00101	0.00021	0.15	0.15	0.0070	0.0045
0275-95	0.007	<0.02	0.020	<0.04	0.0074	<0.006	0.020	0.020	0.00010	0.00010	0.30	0.23	0.0050	0.0060
0276-90	0.003	<0.02	0.008	<0.04	0.0053	<0.006	0.022	0.022	0.00021	0.00019	0.16	0.17	0.0110	0.0035
0277-97	0.004	<0.02	0.022	<0.04	0.0058	<0.006	0.052	0.044	0.00041	0.00029	0.79	0.74	0.0290	0.0195
0278-75	0.009	<0.02	0.019	<0.04	0.0042	<0.006	0.180	0.126	0.00118	0.00082	0.52	0.33	0.1735	0.1700
0279-98	0.010	<0.02	0.020	<0.04	0.0052	<0.006	0.160	0.136	0.00108	0.00078	0.43	0.31	0.0205	0.0065
0281-95	0.012	<0.02	0.016	<0.04	0.0046	<0.006	0.016	0.014	0.00018	0.00014	0.17	0.16	0.0025	0.0025
0282-75	0.003	<0.02	0.015	<0.04	0.0060	<0.006	0.072	0.054	0.00056	0.00032	0.23	0.23	0.0190	0.0120
0283-95	0.004	<0.02	0.010	<0.04	0.0091	<0.006	0.124	0.106	0.00030	0.00027	0.43	0.32	0.0320	0.0250
0284-85	0.003	<0.02	0.005	<0.04	0.0014	<0.006	0.010	0.006	0.00023	0.00011	0.09	0.07	0.0025	0.0025
0285-65	0.003	<0.02	0.011	<0.04	0.0028	<0.006	0.35	0.34	0.00091	0.00101	0.17	0.07	0.0210	0.0135
0286-05	0.007	<0.02	0.014	<0.04	0.0030	<0.006	0.134	0.108	0.00071	0.00037	0.32	0.12	0.0055	0.0045

Appendix 3, continued.

stream -reach	Co		Ni		Cu		Zn		Cd		Ba		Pb	
	T	F	T	F	T	F	T	F	T	F	T	F	T	F
0287-85	0.003	<0.02	0.009	<0.04	0.0032	<0.006	0.028	0.022	0.00037	0.00064	0.05	0.05	0.0080	0.0040
0288-98	0.005	<0.02	0.013	<0.04	0.0026	<0.006	0.30	0.30	0.00064	0.00091	0.45	0.25	0.0090	0.0040
0289-98	0.003	<0.02	0.011	<0.04	0.0021	<0.006	0.066	0.066	0.00025	0.00022	0.43	0.32	0.0030	0.0030
0290-95	0.002	<0.02	0.003	<0.04	0.0011	<0.006	<0.006	<0.006	0.00011	0.00012	0.08	0.03	0.0015	0.0020
0291-50	0.003	<0.02	0.013	<0.04	0.0009	<0.006	<0.006	<0.006	0.00022	0.00019	0.07	<0.02	0.0020	0.0020
0292-98	0.004	<0.02	0.006	<0.04	0.0024	<0.006	0.062	0.062	0.00061	0.00066	0.08	0.05	0.0795	0.0590
0293-99	0.003	<0.02	0.013	<0.04	0.0039	<0.006	0.25	0.23	0.00069	0.00076	0.10	0.07	0.0135	0.0080
0296-90	0.002	<0.02	0.005	<0.04	0.0010	<0.006	<0.006	<0.006	0.00019	0.00014	0.06	0.04	0.0030	0.0020
0300-40	0.003	<0.02	0.004	<0.04	0.0020	<0.006	<0.006	<0.006	0.00014	0.00014	0.11	0.06	0.0030	0.0030
0300-45	0.005	<0.02	0.006	<0.04	0.0012	<0.006	<0.006	<0.006	0.00017	0.00017	0.06	0.06	0.0015	0.0015
0303-90	0.008	<0.02	0.013	<0.04	0.0029	<0.006	0.034	0.026	0.00034	0.00039	0.22	0.20	0.0370	0.0230
0304-95	0.005	<0.02	0.013	<0.04	0.0076	<0.006	0.196	0.182	0.00159	0.00140	0.90	0.70	0.0420	0.0350
0305-01	0.006	<0.02	0.011	<0.04	0.0014	<0.006	0.056	0.048	0.00055	0.00022	0.10	0.10	0.0330	0.0095
0306-95	0.007	<0.02	0.011	<0.04	0.0016	<0.006	0.014	0.010	0.00021	0.00030	0.12	0.13	0.0030	0.0030
0307-98	0.005	<0.02	0.013	<0.04	0.0021	<0.006	0.078	0.078	0.00019	0.00027	0.08	0.13	0.0155	0.0090
0309-95	0.004	<0.02	0.014	<0.04	0.0024	<0.006	0.212	0.208	0.00047	0.00058	0.15	0.20	0.0100	0.0085
0310-90	0.004	<0.02	0.009	<0.04	0.0022	<0.006	0.032	0.016	0.00021	0.00021	0.10	0.12	0.0210	0.0020
0312-90	0.007	<0.02	0.018	<0.04	0.0023	<0.006	0.036	0.024	0.00019	0.00027	0.33	0.30	0.0050	0.0030
0312-95	0.014	<0.02	0.014	<0.04	0.0021	<0.006	0.056	0.048	0.00029	0.00026	0.30	0.26	0.0020	0.0010
0314-98	0.004	<0.02	0.014	<0.04	0.0052	<0.006	0.028	0.028	0.00028	0.00016	0.20	0.18	0.0120	0.0055
0315-98	0.004	<0.02	0.009	<0.04	0.0056	<0.006	0.036	0.028	0.00047	0.00043	0.25	0.28	0.0125	0.0075
0316-90	0.008	<0.02	0.022	<0.04	0.0078	<0.006	0.084	0.060	0.00057	0.00037	0.35	0.28	0.0245	0.0090
0317-50	0.009	<0.02	0.010	<0.04	0.0022	<0.006	0.068	0.052	0.00061	0.00022	0.31	0.27	0.0240	0.0092
0318-95	0.017	<0.02	0.022	<0.04	0.010	<0.006	0.186	0.096	0.00098	0.00050	0.60	0.30	0.0600	0.0095
0319-10	0.009	<0.02	0.024	<0.04	0.0032	<0.006	0.59	0.61	0.00333	0.00332	0.62	0.24	0.0045	0.0015
0320-95	0.007	<0.02	0.026	<0.04	0.0030	<0.006	0.138	0.116	0.00054	0.00040	0.13	0.10	0.0125	0.0075
0321-95	0.005	<0.02	0.005	<0.04	0.0012	<0.006	0.014	0.012	0.00029	0.00016	0.13	0.11	0.0070	0.0035
0354-98	0.005	<0.02	0.008	<0.04	0.0028	<0.006	0.028	0.022	0.00022	0.00009	0.15	0.16	0.0075	0.0030
0355-95	0.008	<0.02	0.012	<0.04	0.0028	<0.006	0.014	0.010	0.00047	0.00031	0.21	0.19	0.0035	0.0025
0356-85	0.008	<0.02	0.020	<0.04	0.0028	<0.006	0.026	0.026	0.00180	0.00063	0.24	0.23	0.0175	0.0045
0357-40	0.003	<0.02	0.010	<0.04	0.0022	<0.006	0.018	0.018	0.00010	0.00013	0.15	0.13	0.0060	0.0025
0357-45	0.004	<0.02	0.011	<0.04	0.0034	<0.006	0.016	0.016	0.00013	0.00006	0.16	0.11	0.0110	0.0040
0358-90	0.005	<0.02	0.010	<0.04	0.0084	<0.006	0.014	0.014	0.00013	0.00020	0.10	0.11	0.0160	0.0120
0359-90	0.005	<0.02	0.009	<0.04	0.0024	<0.006	0.044	0.036	0.00023	0.00015	0.16	0.15	0.0875	0.0505
0360-01	0.009	<0.02	0.070	<0.04	0.027	0.015	0.142	0.076	0.00097	0.00043	0.47	0.13	0.0170	0.0025

Appendix 4. Concentrations ($\mu g\ g^{-1}$) of sodium, magnesium, potassium, calcium, chromium, manganese and iron in 2 cm apical tips and whole plants of Rhynchostegium from intensive survey of 105 sites.

stream -reach	Na tips	Na whole	Mg tips	Mg whole	K tips	K whole	Ca tips	Ca whole	Cr tips	Cr whole	Mn tips	Mn whole	Fe tips	Fe whole
0005-43	371	558	3030	3140	8390	7570	9220	18300	2.93	5.98	2540	14200	4540	8570
0008-07	706	322	1390	1450	7180	5870	13500	25900	3.01	5.79	3120	33100	13300	18100
0008-09	473	378	1450	1620	6460	5940	18100	36200	6.37	9.90	6040	23300	15000	19800
0008-17	358	579	1660	1810	8420	9160	30400	57100	8.77	12.6	1720	6110	8770	11600
0008-40	447	443	1560	1640	6820	7000	24600	47000	4.70	9.40	1480	11900	4420	6910
0008-65	472	351	2580	3040	8760	7520	10100	21600	4.72	7.88	5000	25600	3710	7020
0009-50	544	381	1710	2050	5840	6060	9980	29100	5.14	10.4	1370	5570	5240	8200
0009-65	646	583	2300	2590	7170	6690	10700	19200	5.37	6.91	2010	4370	4610	6910
0009-80	589	322	2840	2880	7850	7020	12600	20800	35.2	43.0	6430	13200	4940	5880
0012-45	394	281	1460	1600	6830	7050	10900	21800	4.17	6.98	1430	6760	6210	8190
0014-14	328	274	2930	2700	6550	4680	8970	12800	5.86	9.36	4480	20800	6270	8770
0015-15	359	453	1760	1750	3890	3210	14500	24400	6.21	9.93	18600	45000	13400	23800
0015-18	392	350	1510	1630	3210	3060	11800	21300	5.67	7.76	9210	30700	10400	17200
0015-98	530	454	1940	1770	6410	5980	13600	18800	5.07	4.65	3440	12200	6940	7970
0024-03	555	230	2770	3340	6600	2340	10400	29000	4.49	8.33	2030	143000	58800	143000
0024-05	734	521	2570	2770	9410	6560	11000	18500	11.2	24.1	4080	32900	27600	41800
0024-20	818	443	2520	2760	9700	8520	11400	22100	5.04	8.75	2870	39700	5920	11700
0024-40	725	493	2620	2580	9660	8470	12100	19400	5.10	5.53	7520	25800	7310	8840
0024-56	666	480	2610	2880	9960	8080	11300	21600	5.85	7.27	5100	28300	7780	13100
0048-90	495	263	1440	1980	5260	4370	10300	17200	3.76	5.80	540	1460	6080	16700
0048-98	400	275	1540	1900	5830	5500	10300	15400	2.50	5.83	433	1080	7000	13400
0055-30	416	478	1290	1320	5060	5420	12500	21400	3.61	3.75	1730	13500	5240	7890
0055-31	300	225	1330	1560	5660	5690	11000	18600	3.92	4.16	412	3780	5620	6940
0061-05	448	240	1940	1920	5070	4220	11400	17400	3.77	5.27	6600	17000	7080	17700
0061-08	461	264	1980	1670	5600	4730	12100	17600	5.93	5.49	4610	25600	8560	22600
0061-50	697	789	2440	2560	7940	7820	10700	18400	3.62	5.67	1600	9110	3620	6070
0081-30	709	328	1780	1990	4690	4920	11600	18900	3.06	4.92	3110	16400	5360	10500
0081-50	237	380	1870	1970	5180	5130	12600	17200	3.30	7.79	5450	22200	7430	12800
0081-95	816	531	2300	2710	9200	8350	11000	19300	8.75	11.7	1700	13200	4300	9320
0085-50	292	471	1370	1560	5040	5550	10200	16800	2.30	5.05	350	719	6580	9260
0085-85	357	354	1520	1720	5800	5360	15400	17600	4.55	6.06	1020	5610	7000	12100
0090-95	232	477	2550	2960	5890	5510	13000	24200	5.78	6.66	3000	14500	8210	11500
0101-05	200	257	1010	1090	3980	4080	11000	16600	1.61	3.59	178	330	6510	9160
0102-85	559	514	1660	1790	5470	6570	10900	26800	6.54	8.98	363	1390	7610	16000
0123-50	430	352	1830	1940	4710	7600	13000	22600	3.54	6.72	8130	63700	4950	11900

Appendix 4, continued.

stream -reach	Na tips	Na whole	Mg tips	Mg whole	K tips	K whole	Ca tips	Ca whole	Cr tips	Cr whole	Mn tips	Mn whole	Fe tips	Fe whole
0166-15	318	320	1380	1340	4170	3490	11600	15100	4.84	5.53	1560	5960	7820	10900
0166-30	416	350	1530	1490	6660	5970	12600	17600	4.72	7.00	2010	7570	5410	8650
0166-40	327	424	1690	1840	5660	6280	12600	21600	5.12	5.83	654	1530	5060	6460
0167-50	437	496	1530	1750	6210	5360	11000	16200	4.95	11.7	1140	12700	6580	19100
0196-35	289	333	1560	1580	4650	4680	10600	17100	3.60	6.48	2510	13500	8200	14900
0196-50	246	288	1570	1570	4740	4610	10800	18700	3.22	6.02	2290	16600	6820	11500
0196-60	494	314	1900	1750	5700	5260	12500	19200	4.31	2.83	874	3070	6210	7080
0196-90	527	368	1790	1790	6110	6080	12600	19800	3.16	3.52	580	2370	5270	6080
0197-50	538	380	1620	1920	5550	4870	13300	19800	3.47	4.70	202	355	4910	9830
0197-90	345	313	1880	1920	4570	4830	17700	26700	2.64	3.85	162	540	2010	4080
0198-95	335	554	1310	1250	5220	5300	15000	27900	4.77	7.80	295	482	5050	7130
0255-10	586	733	1970	1560	12200	10300	3010	3800	4.53	7.46	1330	4340	20900	70600
0255-20	547	524	2340	2190	12100	9020	5470	7960	5.80	8.09	1050	4970	10000	71000
0255-25	861	580	2340	2390	9000	7920	5550	8190	54.0	164	4040	7570	12800	30000
0255-80	669	602	1980	2270	8590	8030	5370	8560	165	254	9330	17900	3430	6960
0258-95	617	608	2640	3230	7550	7360	5390	9310	252	345	7680	12000	12600	20300
0259-50	719	655	2880	3730	8620	6550	8700	11800	647	1330	1980	5030	6830	13600
0261-20	493	579	2630	2520	9860	8320	10200	15800	6.57	9.90	8430	34600	15100	29200
0262-60	527	375	1350	1410	6980	7260	16900	31900	3.41	6.13	4060	8390	1100	2340
0262-65	620	453	1560	1480	9920	7240	11400	20700	6.02	8.39	8680	16800	1450	1800
0265-10	563	595	2770	2820	8390	7480	8140	12800	25.4	41.7	2290	8710	17200	26400
0265-20	699	303	2720	2690	7570	7560	8390	14200	25.6	29.8	4560	30300	8540	15400
0265-30	769	595	2350	2740	8860	6680	8890	17400	62.6	77.6	7170	69500	62600	79600
0274-80	561	649	2720	3350	9520	6810	6220	9790	12.2	16.5	6290	21800	26400	52100
0275-95	672	411	1640	1400	8770	7300	15300	36200	5.72	6.88	7060	19600	2860	3720
0276-90	318	428	1480	1560	4550	4770	12000	20200	3.39	4.19	1370	17300	5610	11500
0277-97	393	625	1560	1620	5710	6910	13500	23900	3.66	4.06	2110	9140	6630	8940
0278-75	744	509	2040	2140	6050	5820	20500	38200	5.47	6.18	477	2960	4540	6180
0279-98	482	509	1780	1860	5180	4890	13100	22600	2.13	3.72	432	2080	2540	3420
0281-95	405	474	1860	2180	5970	6280	10500	28800	3.38	5.85	659	2640	2700	3940
0282-75	360	328	1620	1750	5630	5290	12600	21200	2.19	2.64	240	2010	3230	3750
0283-95	474	514	1490	1890	6100	6970	13100	23500	3.37	4.12	123	726	2080	3430
0284-85	288	285	1710	1760	3650	3450	10700	20200	5.68	7.64	9780	71200	9740	15000
0285-65	375	674	1650	2140	6220	6820	8780	14400	3.11	6.82	887	1800	4980	6960
0286-05	440	650	1580	2060	6330	7690	12100	16300	5.20	6.76	1220	4710	12400	7430

Appendix 4, continued.

stream -reach	Na tips	Na whole	Mg tips	Mg whole	K tips	K whole	Ca tips	Ca whole	Cr tips	Cr whole	Mn tips	Mn whole	Fe tips	Fe whole
0287-85	375	396	1330	2200	4180	4250	11200	21500	3.61	7.92	2280	17000	6980	16600
0288-98	393	456	1400	1460	5060	4960	9540	14800	3.98	5.49	2260	9470	10800	15900
0289-98	314	385	1250	1390	4400	4420	8800	13800	3.04	5.93	325	1340	5240	7890
0290-95	270	336	1850	2100	5410	4790	9820	12700	4.21	7.90	1430	3030	2900	4960
0291-50	358	538	1570	2650	3140	3440	7640	11300	3.33	10.6	3090	11500	3090	7770
0292-98	698	641	3440	3650	6330	4640	7260	10100	10.2	12.2	521	575	8650	9730
0293-99	600	678	2610	2750	5800	4470	7540	10200	7.35	9.49	7160	17600	13300	21900
0296-90	424	376	1800	2220	3720	3340	9180	12500	11.4	2.34	3370	19200	4180	8360
0300-40	364	459	2140	2430	2780	2380	9840	16600	4.81	7.78	4170	23800	7490	10700
0300-45	386	362	1560	2530	3000	3310	10000	15800	4.49	10.4	4030	24100	5530	13500
0303-90	530	328	1640	1720	5070	4420	13700	21800	2.98	3.58	559	9380	2910	6100
0304-95	438	434	1310	1260	6600	5440	13200	21900	2.39	5.20	108	572	3070	9460
0306-01	229	426	1330	1410	4700	4920	12900	19600	1.61	2.94	207	612	1160	3000
0306-95	410	329	1380	1190	3320	3680	14900	24700	5.07	5.96	7050	33800	11500	15800
0307-98	435	463	1580	1990	4310	4840	14900	26600	3.48	4.76	730	16600	6090	7790
0309-95	515	485	1560	1780	5110	5780	14400	23300	4.88	5.19	855	5110	4620	6740
0310-90	183	442	1000	1480	3420	5300	8250	18600	2.77	4.42	889	3570	5210	6260
0312-90	622	373	1840	1890	7140	6480	19100	30400	2.81	5.51	384	4810	4430	6260
0312-95	283	258	1460	1630	5160	5260	10100	16400	3.50	5.79	441	4000	7740	11600
0314-98	270	233	1300	1420	5700	5530	14400	24300	3.00	3.00	335	1170	1920	2840
0315-98	372	336	2070	1860	5790	4870	12400	20400	4.02	4.87	1610	14700	5610	6440
0316-90	442	283	1880	1840	5540	4290	13500	20400	3.04	2.91	295	689	3570	3520
0317-50	620	432	1420	1400	6500	5940	17600	27900	3.07	3.60	1000	2920	1740	3780
0318-95	459	356	2010	2560	5260	5310	20800	39500	4.49	6.64	841	2720	6930	8140
0319-10	506	434	853	756	3070	3290	9920	14600	3.77	4.73	139	279	38200	60800
0320-95	449	488	1740	1690	7780	7140	28300	27800	14.8	38.2	3420	17800	14400	12400
0321-95	322	340	1720	1950	4290	4620	12100	23000	4.76	6.52	4340	39100	5040	7880
0354-98	504	660	1980	1890	8900	8050	12200	20600	5.93	5.28	4270	14600	4350	8560
0355-95	350	320	1450	1710	5510	5160	11400	27900	2.15	5.33	2760	51200	4720	19600
0356-85	532	465	1980	2380	8810	8840	13000	22600	1.98	3.49	372	4070	2000	3020
0357-40	570	680	1740	1740	7930	8890	10900	17100	1.65	3.66	3060	8020	1680	3140
0357-45	818	375	1850	1720	6590	7500	10900	16700	1.71	4.68	890	9660	1290	3440
0358-90	388	282	1660	1480	3550	2950	13400	21500	6.66	11.4	7600	40300	7770	19100
0359-90	623	521	2600	2290	8840	7010	15200	23000	7.42	6.62	742	2480	6790	8960
0360-01	481	476	2170	1950	4860	8580	12500	19700	4.44	6.12	898	4630	2520	6120

Appendix 5. Concentrations ($\mu g\ g^{-1}$) of cobalt, nickel, copper, zinc, cadmium, barium and lead in 2 cm apical tips and whole plants of <u>Rhynchostegium</u> from intensive survey of 105 sites.

stream -reach	Co		Ni		Cu		Zn		Cd		Ba		Pb	
	tips	whole	tips	whole	tips	whole	tips	whole	tips	whole	tips	whole	tips	whole
0005-43	7.22	41.8	20.5	47.8	12.8	23.9	87.4	169	1.17	0.498	156	337	14.6	31.9
0008-07	20.5	201	22.0	66.0	10.5	16.9	1660	7880	5.44	0.885	249	1050	370	1760
0008-09	50.6	171	30.9	64.8	19.7	28.8	2440	5850	8.24	24.3	328	844	1100	2300
0008-17	20.0	112	38.5	28.1	37.6	55.5	2150	3060	7.70	8.20	237	536	707	1140
0008-40	22.9	142	34.7	66.0	23.5	28.4	1300	3540	8.72	16.8	201	573	324	886
0008-65	23.0	122	29.6	104	14.9	22.9	354	1620	1.80	7.30	202	716	87.6	244
0009-50	7.46	28.2	9.77	21.7	12.5	15.9	468	880	2.92	5.18	302	652	252	635
0009-65	14.3	17.0	12.9	18.2	10.9	15.3	301	448	1.28	2.59	288	517	178	367
0009-80	21.4	44.2	28.9	54.3	26.1	31.7	433	871	6.63	13.8	474	707	207	362
0012-45	28.1	150	98.4	157	87.8	99.7	4390	7190	8.69	15.0	62.1	178	842	1370
0014-14	18.2	56.5	31.0	146	10.9	14.0	172	566	1.49	5.04	402	1040	25.3	42.4
0015-15	63.4	224	59.6	121	11.1	14.9	1060	1890	8.40	17.1	943	2060	974	4460
0015-18	31.6	134	36.8	85.2	12.4	16.6	1180	2680	8.50	21.1	477	1340	916	2520
0015-98	16.7	41.2	28.3	54.3	19.2	20.7	2000	3270	4.32	17.6	306	599	955	1750
0024-03	19.7	542	30.4	694	39.6	113	224	2730	2.38	23.4	119	1120	42.3	120
0024-05	32.7	257	42.6	277	45.2	75.9	834	2650	3.64	9.78	157	510	125	190
0024-20	18.8	288	31.5	201	27.7	46.0	3150	11800	12.3	27.6	157	585	83.1	135
0024-40	60.0	230	51.0	152	29.5	36.8	2820	6310	3.76	8.66	215	537	77.8	72.8
0024-56	29.0	162	39.8	167	24.4	36.4	1900	5360	1.99	7.38	199	674	69.7	73.8
0048-90	7.02	12.7	37.6	53.2	17.8	34.1	8840	13500	33.8	46.4	815	546	840	1770
0048-98	7.75	10.5	35.0	47.8	16.7	26.7	7370	11700	30.0	47.8	279	674	842	1640
0055-30	12.8	120	18.1	49.4	9.02	27.9	1060	3590	4.89	19.9	120	382	695	2770
0055-31	6.16	21.3	22.0	42.3	8.66	20.8	2460	4440	13.9	21.5	108	347	525	1500
0061-05	26.3	92.8	24.9	55.6	7.90	11.6	536	1070	3.42	7.29	265	612	75.5	278
0061-08	55.2	409	43.5	106	15.6	24.7	5350	9740	14.2	47.3	173	592	988	4630
0061-50	7.81	32.6	16.2	55.3	9.34	18.2	648	1770	2.23	7.15	362	1180	64.1	310
0081-30	11.4	63.3	37.8	72.6	10.0	16.4	2300	6680	8.57	29.3	143	488	459	1300
0081-50	18.5	96.2	35.2	64.1	10.8	16.4	3520	8660	12.1	40.3	248	802	473	1280
0081-95	16.0	53.8	20.3	40.1	13.0	19.4	1560	4500	5.49	18.3	208	699	166	444
0085-50	5.74	8.16	22.4	40.4	9.28	17.7	7250	7910	26.5	32.0	252	379	327	488
0085-85	8.65	22.8	26.4	28.8	15.0	16.1	3480	7080	10.9	28.3	223	1300	285	374
0090-95	20.7	74.4	33.1	173	30.6	43.6	470	1730	3.51	10.8	198	1090	487	806
0101-05	4.66	4.58	25.8	26.8	4.74	9.02	4560	4720	21.6	26.1	178	126	1620	5700
0102-85	10.2	17.3	36.9	52.6	25.0	38.7	3270	5730	21.9	25.5	89.2	228	2350	5880
0123-50	25.5	214	28.6	174	9.90	15.2	283	1850	1.65	17.4	554	1750	25.9	53.8

Appendix 5, continued

stream -reach	Co tips	Co whole	Ni tips	Ni whole	Cu tips	Cu whole	Zn tips	Zn whole	Cd tips	Cd whole	Ba tips	Ba whole	Pb tips	Pb whole
0166-15	24.6	54.8	17.1	28.4	8.35	11.1	598	1200	4.55	12.5	332	590	79.7	153
0166-30	25.4	66.6	11.7	29.3	11.1	14.5	888	2090	9.30	26.8	1800	3230	833	1940
0166-40	9.37	16.9	11.5	17.5	10.6	15.1	626	978	5.77	9.50	1960	2450	501	849
0167-50	9.05	49.1	15.6	51.2	14.2	110	2320	16300	15.0	67.5	132	423	2640	17800
0196-35	22.5	112	15.0	34.2	7.30	10.2	275	797	2.28	9.64	427	1780	134	522
0196-50	21.0	144	11.6	33.3	8.05	10.5	551	1820	3.98	26.9	1120	1220	313	615
0196-60	12.3	23.3	17.4	24.3	11.3	10.0	868	1340	10.8	17.5	3270	1210	456	520
0196-90	9.07	24.1	15.6	20.5	9.17	8.84	617	1060	9.28	16.0	1130	1200	327	493
0197-50	6.24	9.23	30.5	41.9	13.6	19.7	3470	5170	18.0	22.2	1034	1440	1960	4150
0197-90	5.48	5.66	22.5	36.2	9.24	15.8	1980	3130	13.2	19.6	964	2200	842	1820
0198-95	6.13	10.4	20.4	38.5	21.0	31.8	1870	2940	13.8	25.0	2730	2940	2840	6330
0255-10	22.9	52.6	19.7	33.0	18.4	25.9	293	292	3.20	5.43	133	159	58.6	125
0255-20	12.1	48.6	14.1	29.4	20.3	30.5	2130	2890	2.68	4.24	134	183	33.5	155
0255-25	27.8	60.3	32.6	36.0	29.0	42.3	1540	2420	2.57	9.01	193	370	69.4	160
0255-80	42.8	87.9	18.2	32.0	19.0	29.4	1250	2130	1.16	4.28	248	463	54.5	88.3
0258-95	46.1	97.1	32.0	48.0	42.8	52.5	617	923	7.05	10.5	340	456	146	263
0259-50	11.7	24.4	25.9	37.3	82.6	121	755	1040	64.7	82.5	180	235	1920	3810
0261-20	25.8	188	31.5	168	42.3	71.3	482	1530	3.50	18.2	712	1790	37.2	91.1
0262-60	12.2	24.4	24.0	46.8	23.4	29.0	170	306	1.46	2.90	162	437	26.0	37.1
0262-65	30.5	53.2	29.8	72.4	14.5	34.6	372	1140	4.25	10.5	230	446	42.5	47.7
0265-10	24.6	59.5	44.2	114	30.3	40.5	429	779	6.87	14.7	134	292	135	270
0265-20	25.0	224	68.0	378	34.6	58.7	660	2450	10.9	47.8	214	664	113	318
0265-30	44.8	254	44.3	374	30.2	56.2	352	1500	4.69	20.4	183	1060	102	139
0274-80	45.7	170	44.9	102	116	157	451	1040	20.1	24.5	459	1040	306	553
0275-95	11.8	28.0	28.2	49.1	37.7	48.4	243	330	2.92	4.14	183	468	105	182
0276-90	14.8	156	18.0	40.9	6.67	8.64	529	1760	3.28	23.0	465	2100	203	954
0277-97	9.27	39.4	18.3	22.6	9.48	12.5	706	1570	6.36	18.7	1850	2880	636	1240
0278-75	7.44	11.7	20.9	38.2	27.9	32.7	1740	2730	31.4	41.8	3000	3140	4980	9050
0279-98	8.74	11.5	27.9	43.1	13.4	15.8	2130	3380	23.4	39.1	2230	2370	660	3350
0281-95	4.84	13.7	9.57	21.4	10.0	13.8	67.6	128	1.24	2.34	141	385	16.9	30.9
0282-75	2.19	31.2	14.3	22.5	3.86	5.38	553	1300	5.95	13.4	913	1790	230	640
0283-95	2.72	4.80	16.5	18.1	8.43	13.0	921	1690	2.59	5.83	584	1340	376	1790
0284-85	48.7	494	46.2	247	7.79	11.8	495	3180	3.81	27.7	608	2650	114	247
0285-65	10.8	20.7	38.4	73.8	18.1	34.4	3840	7660	17.6	21.1	206	309	1680	2510
0286-05	12.2	25.7	19.2	23.9	10.7	13.5	2820	4580	9.49	18.2	102	503	138	265

stream -reach	Co tips	Co whole	Ni tips	Ni whole	Cu tips	Cu whole	Zn tips	Zn whole	Cd tips	Cd whole	Ba tips	Ba whole	Pb tips	Pb whole
0287-85	24.6	169	15.2	54.0	7.98	17.3	499	2120	2.94	15.1	114	465	166	814
0288-98	26.3	72.6	32.5	55.8	10.3	31.9	4790	22300	17.2	53.1	931	443	777	1950
0289-98	4.40	10.8	10.0	17.4	6.02	9.78	572	896	2.37	4.36	406	764	120	271
0290-95	8.62	12.7	14.8	17.7	15.4	19.3	346	534	4.21	7.40	200	280	95.2	202
0291-50	8.43	26.6	17.6	39.7	11.4	18.5	549	1200	10.2	23.0	269	460	112	344
0292-98	16.0	13.0	15.6	16.4	59.6	82.9	912	973	10.0	12.5	903	1130	8690	11100
0293-99	102	383	40.8	86.2	25.0	32.7	3680	6180	19.3	43.9	212	416	310	1040
0296-90	8.72	51.8	17.7	56.8	9.18	13.7	494	1610	13.4	47.6	407	766	44.2	142
0300-40	18.4	59.4	24.8	83.2	10.5	15.4	348	1580	4.06	25.9	225	710	38.5	97.2
0300-45	13.6	34.8	14.6	47.1	9.10	13.3	397	1520	3.57	20.9	236	781	56.4	161
0303-90	9.70	51.1	14.2	33.6	6.12	8.92	626	1800	7.46	42.0	462	238	2010	5190
0304-95	4.89	8.51	22.9	32.3	9.78	21.3	1430	8200	16.2	52.8	3550	2400	705	4260
0305-01	7.30	5.82	50.7	126	5.88	16.2	291	567	1.67	2.22	80.4	187	680	1580
0306-95	30.8	237	23.4	42.1	11.0	14.8	341	1090	2.67	9.12	392	1510	323	754
0307-98	3.56	221	28.2	64.9	9.04	14.3	1240	5490	4.97	22.5	74.6	433	680	2250
0309-95	4.88	17.6	21.8	51.9	8.93	13.3	1260	3190	4.43	12.0	279	324	1350	3800
0310-90	5.06	17.3	22.4	53.0	5.01	10.1	300	679	2.06	5.22	103	370	118	193
0312-90	7.03	25.6	13.7	27.4	6.27	9.18	454	1310	2.49	8.10	324	700	89.7	248
0312-95	1.83	15.9	15.8	22.2	7.74	12.8	1330	2080	2.92	8.95	354	658	175	316
0314-98	4.30	11.9	12.7	16.7	4.90	6.87	575	616	1.90	3.32	240	418	170	348
0315-98	15.2	143	18.7	45.1	9.45	10.3	1090	3330	28.3	89.5	1040	1420	532	1170
0316-90	4.38	11.6	9.47	13.8	5.90	6.43	893	1140	6.70	9.11	482	842	429	681
0317-50	12.6	16.5	15.0	21.9	5.67	9.00	1100	1790	6.97	11.2	768	1490	661	1770
0318-95	9.27	15.0	22.6	33.2	22.0	25.9	2150	3080	17.2	21.7	1860	1360	1540	2400
0319-10	8.53	15.6	34.7	51.3	16.4	23.7	2330	3520	16.6	27.6	322	408	3760	5780
0320-95	80.5	497	80.5	140	30.8	32.5	4730	8610	11.1	28.0	225	608	1900	2850
0321-95	12.0	118	75.6	362	11.2	17.9	630	3940	3.26	26.3	326	1610	448	1580
0354-98	20.4	35.2	18.8	29.2	10.3	13.3	564	2320	2.57	11.3	474	1480	63.3	100
0355-95	4.02	40.4	9.90	42.5	4.95	10.3	69.1	549	1.21	7.83	327	2880	31.8	111
0356-85	3.80	11.3	14.4	53.5	6.99	10.7	140	436	1.37	3.60	228	565	28.9	142
0357-40	10.7	18.6	10.4	13.9	8.26	8.72	127	357	0.661	1.92	182	472	81.0	139
0357-45	7.91	11.9	31.6	18.2	6.07	9.10	270	353	0.264	6.77	132	502	35.6	68.9
0358-90	33.5	255	22.3	59.1	9.76	16.1	560	2480	6.99	22.8	621	1320	632	2870
0359-90	3.95	15.8	23.2	36.0	21.5	31.2	766	1070	9.94	14.7	276	661	3440	6810
0360-01	11.3	24.7	60.2	141	43.4	50.1	666	1260	5.07	6.37	132	265	53.9	111