Potential Toxic Effects of Bracken (*Pteridium aquilinum* (L) Kuhn) on Invertebrates and Diatoms in Welsh Upland Streams

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy by Pavel Toropov

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THESIS CONTAINS
CD/DVD

"...it sounds like a formula for some deadly gas.

Will you listen! This is not funny to me.

Perhaps he should be a Welsh miner, or a biologist, or a young Scots doctor. Someone from quite another background, bringing another point of view..."

Eric Newby
A Short Walk in the Hindu Kush

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Abstract

Bracken fern (*Pteridium aquilinum* (L.) Kuhn) is an invasive plant, which has taken over large tracts of upland land in the United Kingdom. It is a species of vast toxic potential, producing and retaining a great variety of toxic secondary metabolites. Animals feed on bracken when other resources are limited, which leads to a variety of diseases including a range of cancers. Laboratory administration of bracken extracts to animals has led to identification of numerous mutagens and toxins contained in this plant.

The hypothesis of this study was that bracken exerts toxic effects on the upland streams. Many small streams in the uplands have substantial bracken cover in their catchments. Bracken run-off has been shown to be toxic to plant and animal species, with many toxins being water soluble, including the main carcinogen, ptaquiloside. The uplands have high precipitation and bracken stands intercept large amounts of rain with their closed canopy, allowing for a possibility of leaching of toxins into the streams. Also, feeding on bracken may be taking place by invertebrates in upland streams. Contamination of drinking water supplies by bracken toxins had previously been put forward as one of the possible reasons behind high rates of gastric cancer in North Wales.

A two-stage approach was used in this study. A survey was conducted where invertebrates and diatoms were sampled from two groups of streams in North Wales, which differed in the amount of bracken in the catchment. Physiochemical variables known to influence the invertebrate and diatom communities, and total amount of bracken in the catchment were measured for each of the study streams. Direct gradient analysis methods were then used to see if bracken was an important variable in explaining variation in the data. Neither for the diatoms nor invertebrates did the amount of bracken emerge as significant, but the density and length of fronds did in the invertebrate dataset, with almost all species associated with low frond density and length. These two variables, however, were correlated with Fe levels, which were more likely to be the causative factor. Bracken biomass was not correlated with the abundance of shredders in the streams. The stream water was tested on one occasion for ptaquiloside, the main bracken carcinogen, with no traces found in any of the study streams.

Many studies on bracken toxicity used destructive extraction methods and unrealistic dosage with little relevance to natural interaction of bracken with the biota. For this reason the second part of the study involved an experiment, where the growth and mortality of *Gammarus pulex* subjected to run-off from fresh, undamaged bracken fronds was monitored. There was no increase in mortality compared with the control group, with no differences in growth rate. It appeared that without destructive treatment, such as maceration, the toxins were locked in bracken fronds without being leached out. The run-off was tested for ptaquiloside, with negative result, but run-off from crushed fronds was found to contain this toxin.

The results suggest there being no toxic effects exerted by bracken on upland streams. Bracken may, however, play a role in the ecology of the running waters in the uplands by affecting the composition and amount of the allochthonous organic matter in the streams.

CHAPTER 1

Introduction to the research topic: British uplands, biology and toxicity of bracken, the link between bracken and running waters in the uplands

1. Introduction to the research topic

British uplands have undergone extensive ecological changes as the result of human activity. Upland freshwaters have been severely affected by acidification and terrestrial vegetation changes with deleterious effects on flora and fauna. One of the consequences of human activity in the uplands has been the emergence of bracken fern (*Pteridium aquilinum*) as an invasive plant, which can dominate upland vegetation, often completely excluding other plants over large areas. Bracken is an extremely toxic species, containing a great variety of highly toxic metabolites. In some catchments of upland streams bracken is the dominant plant and intercepts a large proportion of precipitation. Bracken fronds are also transported into the stream, especially in autumn when senescent. Many bracken toxins are water soluble, and can be removed from fronds by water, and potentially by rain. There exists, therefore, a potential for bracken to exert toxic effects in the streams, via leaching of toxins from its fronds by rainwater or by stream invertebrates feeding on bracken fronds which end up in the stream. There have so far been no attempts to assess bracken toxicity to aquatic animals and its role in the ecology of upland streams.

1.2 Study objectives

The aim of this study was to see if bracken influences invertebrate and diatom communities in upland streams in Wales. The project comprised two parts, the first being an extensive survey of invertebrate and diatom communities in bracken - infested streams in North Wales. The survey used multivariate statistical methods of direct gradient analysis. The second part was an experiment where the growth and mortality rates of *Gammarus pulex* were examined in response to bracken run-off.

1.3 Thesis layout

Chapter 1 introduces the research topic. Chapters 2 & 3 present the results of the stream survey. In Chapter 2 the study streams are compared in terms of their physiochemical variables. In Chapter 3 these physiochemical variables of the study catchments are used in direct gradient analysis of invertebrate and diatom species data in order to see if the bracken characteristics of study catchments are important in explaining variation in the species data. Chapter 4 presents the results of the bracken run-off experiment. Chapter 5 includes the summary and the conclusions of the study.

1.4 British uplands

Uplands are usually defined as areas above the upper limit of enclosed land (Ratcliffe, 1977). In the UK, where the natural ecosystems have been so greatly modified by man, the uplands are the last remaining extensive semi-natural habitat (Ratcliffe & Thompson, 1988). The largest upland areas are in Scotland, northern England and Wales, and small areas of south - west England (Birks, 1988). The history of uplands is that of continuous ecological change, which in the past 5000 years has been greatly accelerated by human activity, so that the present upland landscape is largely man-made.

1.4.1 Vegetation

The modern British upland landscape is an open and wind-swept mosaic of different vegetation types - grasslands, heaths, peat bogs and bracken, with occasional patches of deciduous woodland and orderly plantations of conifers (Fig.1). Upland vegetation in the UK is divided into submontane and montane zones (Birks, 1988). The former is between the enclosed farmland and the potential tree - line, and the latter applies to the vegetation lying above the potential tree-line. There is a great overlap in terms of plant communities between the two zones, but the latter includes several species of alpine plants occurring neither in the submontane zone below, nor anywhere else in the UK.

The submontane zone, often refered to as moorland, is again a vegetation mosaic. Description of vegetation of the submontane zone is based on Backshall (2001). The dominance of each vegetation type varies geographically in the UK, as well as locally. Heath communities, both wet and dry, are common and are of European conservation significance. Dry heath is dominated by dwarf shrubs, mainly heather (*Calluna vulgaris*), but also bilberry (*Vaccinium myrtilis*), and crowberry (*Empetrum nigrum*). This vegetation type occurs on mineral soils with humus-rich surface horizons. Dominant plants of the wet heaths are also dwarf ericoid shrubs, but underlying soils are peat down to the depth of 0.5 metres. Bryophytes are abundant in wet heath, especially *Sphagnum spp.* Other plant species may include purple moor-grass (*Molinia caerulea*), heath rush (*Juncus squarrosus*), deer grass (*Trichophorum cespitosum*), and matt grass (*Nardus stricta*).

Another common upland plant community lies in mires. These are waterlogged habitats which occur on peat. There are several types of mires, for example raised mires or bogs, which derive water and nutrients from atmospheric precipitation, and soligenous mires,

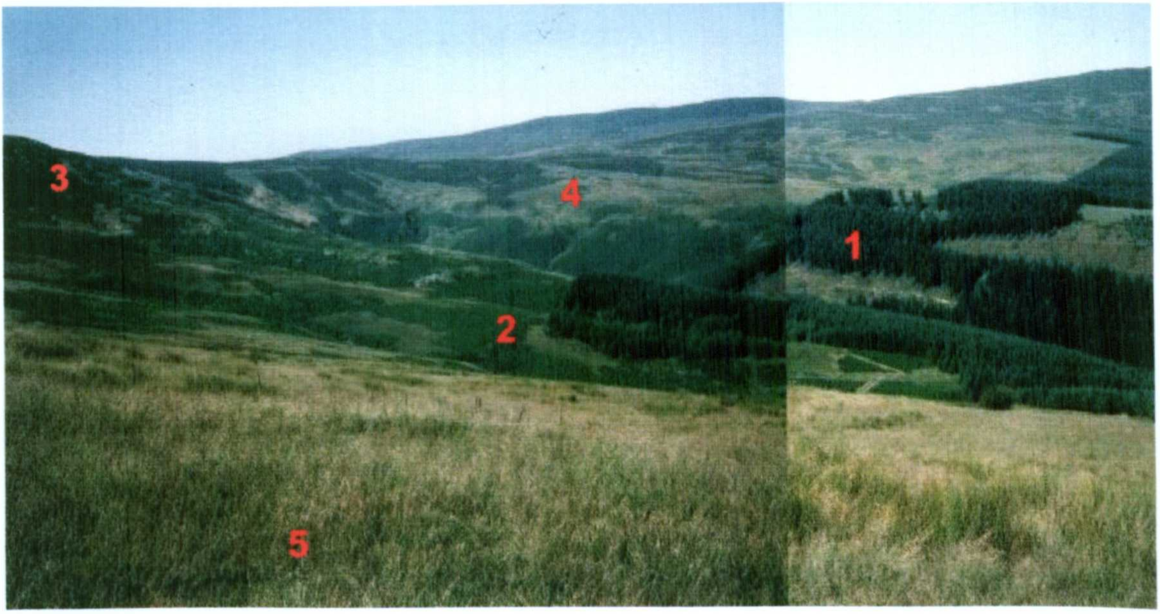


Fig.1 Upland vegetation types in the Berwyn, North Wales. 1 plantation of exotic conifers; 2 bracken; 3 *Calluna* mire; 4 *Molinia* mire; 5 *Nardus stricta* and *Juncus squarrosus* grassland.

which are associated with ground or flowing water. The vast majority of upland mires are blanket mires, with vegetation mainly of heather, cottongrass (*Eriophorum vaginatum)*, deergrass (*Trichophorum cespitosum*) and several species of *Sphagnum*. Plants characteristic of soligenous mires include *Sphagnum*, *Molinia*, sedges (*Carex*) and rushes (*Juncus*). Upland grasslands can be divided into acidic, calcareous and neutral. The first type is the most extensive and is the product of heavy grazing on dwarf shrub communities. Typical species include sheep's fescue (*Festuca ovina*), *Agrostis*, mat grass (*Nardus stricta*), and moorgrass species (*Molinia spp.*).

Woodland was the original vegetation cover over most of the submontane uplands, but virtually none of the remnants of the original woodland have survived. The isolated fragments that are present are either *semi-natural* - composed primarily of native vegetation produced by natural regeneration, coppice or stump regrowth or *plantation* - primarily non-native conifers, such as sitka spruce (*Picea sitchensis*), and, to a lesser extent, native broadleaved species such as *Quercus spp.* (Ratcliffe & Thompson, 1988).

1.4.2 History of upland vegetation change

The postglacial (after 10000BP) history of submontane upland vegetation in Britain is that of the transition from the original woodland to the moorland of today. This change has been reconstructed from pollen and macrofossil analysis.

Most of the uplands was forested after the last glaciation - it is thought that only the highest areas of North Wales, the Lake District and Scottish Highlands were above the tree-line (Birks, 1988). The species composition of the wildwood varied geographically. In North Wales the woodland species composition was *Quercus*, *Betula*, *Corylus* and *Ulmus glabra*, with some *Pinus* on poorer soils (Birks, 1988). This vegetation existed from 7000-5000BP prior to intensive human impact. The first small local woodland clearances by Neolithic and Bronze age people occurred about 5000BP (Birks, 1988). These clearances for arable and grazing land were temporary, the ecologically important permanent large-scale deforestation occurred later, and at different times in different upland areas. The uplands of North Wales were cleared approximately 2100 years BP (Walker, 1978).

1.4.3 Soils

Base - deficient, acidic soils such as podzols, semi - podzols, rankers and peats are the main soil types of the modern uplands (Miles, 1988). In the cool, moist climate of the uplands leaching of exchangeable ions is a natural process, often accompanied by acid humus accumulation (Ratcliffe & Thompson, 1988). It is thought that acidic soils in uplands had already developed by 8000BP (Birks, 1988). Blanket mires have developed naturally as the result of this process, as accumulation of mor and formation of iron pans decreased soil aeration and vertical drainage of water, leading to waterlogging (Ratcliffe & Thompson, 1988). Subsequent anthropogenic involvement also contributed to and accelerated bog formation and soil acidification, acting together with climatic changes. Overall, however, the anthropogenic processes were of secondary importance in acidification of upland soils (Ratcliffe & Thompson, 1988).

1.4.4 Recent ecological changes in the uplands - management and agriculture

Most intensive agriculture in Britain takes place in the lowlands, with the upland areas used primarily for livestock rearing. Intensive sheep grazing in most upland areas started in the Middle Ages (Backshall, 2001). Increase in grazing and burning has further altered the vegetation: large tracts of submontane heaths of heather, *Vaccinium myrtilus*, *Erica cinerea*, *E. tetralix* and *Empetrum nigrum* were converted to acid grasslands. *Festuca ovina*, *Deschampsia flexuosa*, *Nardus stricta*, *Juncus squarrosus* and *Molinia caerulea* became dominant on well-drained soils, and *Eriophorum vaginatum*, *E. angustifolium* and *Molinia caerulea* on wetter soils. Most of the dry *Salix - Dryas* heaths were converted to species-rich grassland and tall herb communities and scrub retreated to inaccessible crags and cliff ledges (Ratcliffe & Thompson, 1988). Increased grazing also prevented any regeneration of woodland and allowed bracken expansion.

The nineteenth century brought the practice of managing heather moors for grouse shooting (Backshall, 2001). This policy affected the uplands primarily through increased burning, which was carried out to produce a mosaic of heather stands of different ages. This resulted in exclusion of other plants from heather stands and reduction in areas of older heather (Gimingham, 1995). Composition of terrestrial invertebrate communities was also altered (Backshall, 2001). Additionally, moor drainage was practised widely to improve the heather cover for the grouse, leading to the loss of wet habitats and increase in peat erosion, which in turn affected freshwater habitats (Harding & Bell, 2001). Intensive persecution of predators as part of gamekeeping and sheep rearing resulted in drastic reductions in populations of predatory birds such as the golden eagle (*Aquila chrysaetos*), red kite (*Milvus milvus*), raven (*Corvus corax*) and peregrine falcon (*Falco peregrinus*) (Ratcliffe & Thompson, 1988).

Industrial acidification was the latest human impact on the uplands, beginning with the start of the industrial revolution and continuing to the present day. The effects of acidification on freshwaters are reviewed in Chapter 3; it has also lead to local loss of *Sphagnum* moss, increase in peat erosion and spread of *Molinia caerulea* (Ratcliffe & Thompson, 1988).

Modern uplands remain a managed ecosystem. Grazing, burning and cutting of vegetation, bracken and rhododendron management, conversion of semi-natural vegetation to improved pasture, and in places, predator control, are all practised as part of agricultural and sporting management (Backshall, 2001). Grazing, primarily by hill sheep, is the main process controlling the dynamics of upland vegetation, maintaining the shrub-grassland dominance and preventing succession to woodland. High grazing pressure leads to the spread of grasses, rushes and sedges and establishment of bracken at the expense of shrubs. Low grazing pressure allows invasion of trees and shrubs and regeneration of woodland. These vegetation changes are then followed by a change in the vertebrate and invertebrate fauna (Backshall, 2001). It is, therefore, crucial, to regulate and maintain grazing regimes so as to preserve the desired local plant communities. This brings conservation in the uplands in conflict with the needs and objectives of the farmers whose objectives are of increased yield, rather than of conservation, but who at the same time manage the mechanisms regulating upland ecosystems. Recent trends in upland livestock practices include greater density of sheep on pastures and all year round grazing, made possible by the introduction of supplementary feeding, a decline in shepherding (dispersal of sheep over a wide area), and conversion of vegetation to improved pastures (Backshall, 2001).

1.4.5 Ecological changes in upland streams

Upland freshwaters are primarily nutrient-poor, being situated in acidic soils of low ionic strength. The majority of running waters in the uplands are small, often first order, streams. The ecology of freshwaters is closely connected with the ecology of their catchments (Hynes, 1970). The enormous changes in terrestrial upland ecology over the past 5000 years have had a great impact on the running waters in the uplands. The effects of modern day logging in North America may reflect the processes that took place several thousand years ago in Europe.

Many stream ecosystems are heterotrophic - most of their energy originates from outside the system, in the surrounding land, and is imported into the stream in the form of either dissolved (DOM) or particulate organic matter (POM). Fisher & Likens (1973) showed that 99% of the total energy input for a first order woodland stream in New Hampshire, USA was allochthonous (of external origin). Only 1% of energy was autotrophic, the product of stream photosynthesis. The heterotrophy / autotrophy ratio is a common criterion used in comparing stream communities. This ratio is controlled by organic matter inputs, light, temperature, and flow regime of the stream (Fisher & Likens, 1973) all of which are affected by terrestrial vegetation. In deciduous woodland the leaf litter is the primary source of coarse particulate organic matter (CPOM) to the streams. Invertebrates feeding on leaves (shredders) invade the leaf litter and through feeding reduce leaves to fragments, making this resource available to collectors and filter-feeders (Cummins *et al.*, 1973). Terrestrial vegetation varies in quality as a food resource (Kaushik & Hynes, 1971). Leaves with high content of tannins and lignins as well as those with a hard or hairy cuticle are less susceptible to colonisation by fungi and as result are less palatable to shredders (Allan, 1995).

In the British uplands native deciduous trees were replaced with shrubs and grasses. The quantity of allochthonous inputs was therefore reduced with the loss of tree cover. This has been observed in the modern day North American streams after logging (Webster *et al.*, 1992). The quality of organic matter is also reduced by deforestation, as fibrous grasses, ferns and shrubs are a poorer food resource than leaves of deciduous trees. They would take longer to become conditioned by microbes and fungi, thus requiring longer residence time in the stream to become palatable. This decline in food supply is increased by other effects of deforestation - increased streamflow and greater frequency of high discharge events in deforested areas (Keppeler & Ziemer, 1990; Wright *et al.*, 1990), and the reduction in the amount of woody debris which retain the organic matter in the stream (Ward & Aumen, 1986). Food for shredders in deforested streams is therefore scarcer and of poorer quality and is rapidly flushed out as the streams lose their retentive capability. Another consequence of the removal of woodland cover is reduction in shading, especially during summer, which causes water temperature to increase (Swift & Messer, 1971). Erosion is increased as the tree cover is lost and the transport of sediment into the stream is increased. This is known to influence macroinvertebrate abundances further downstream (Webster *et al.*, 1992).

The smaller the stream the more it is influenced by terrestrial vegetation - the greater is its shading by trees, its dependence on the autumnal supply of leaves, and the more its flow regime is affected by rainfall. Ross (1963), surveying distributions of caddisflies in USA streams, discovered that the invertebrate fauna of small streams was more rigidly confined to terrestrial biomes than that of larger streams. In the British uplands the streams are small, many of first order, and the effects of deforestation on their ecology are likely to have been extremely extensive.

Review of logging effects on streams in the United States by Webster *et al.* (1992) suggests that a decrease in the amount of organic matter of terrestrial origin, combined with reduction in shading, resulted in an increase in in-stream photosynthesis by periphyton. There was a corresponding reduction in the number of shredders and an increase in the number of grazers and collectors that feed on algae. In the U.K., however, the dominance of acid tolerant species has overshadowed ecological changes that had taken place several thousand years before. It is therefore difficult to distinguish the effects of acidification from the legacy of earlier deforestation.

1.5 Bracken

Bracken fern (*Pteridium aquilinum* (L.) Kuhn) is one of the most successful plant species on the planet. It has a truly global distribution, being present on all continents, except Antarctica, absent only from the tundra and the desert regions (Taylor, 1990). It is thought to have first evolved in the tropics during the Jurassic period and then spread into the temperate regions (Page, 1989). Taxonomy of the genus *Pteridium* is extremely confused, due to variable ecology and morphology of bracken, and is subject to perpetual revision and debate. Bracken found in the U.K. is predominantly *Pteridium aquilinum* subspecies *aquilinum*. Bracken is highly toxic and carcinogenic, and a tremendous competitor and invader. World-wide, and particularly in the U.K. bracken now covers extensive areas of land, often to almost complete exclusion of other plants (Fig. 2 & 3). It has become a pest species, interfering with agriculture, forestry and farming, harbouring reservoirs of ticks, which carry Lyme disease, it poisons livestock and may be a factor behind high rates of human cancer in some areas.

Fig.2 Bracken infestation in the valleys on Eastern side of the Clwydian Range, North Wales. Iron Age fort in the background.

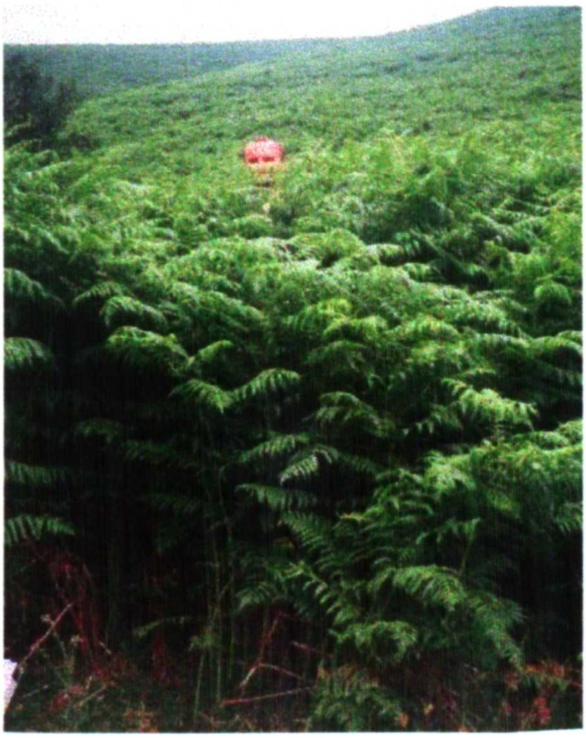


Fig.3 Bracken fronds by the stream in the above valley.

1.5.1 Morphology and reproduction

Bracken consists of the visible fronds and an underground stem system, the rhizome, which accounts for the overwhelming proportion of the plant's biomass. The rhizome is responsible for the vegetative spread of the plant, and acts as a reserve of nutrients and carbohydrates, whereas the fronds are the photosynthesising part of the plant and export carbohydrates to the rhizome, where they are then stored. Watt (1940) described the structure of the rhizome, and identified the long shoots, which formed the framework of the rhizome and acted as carbohydrate stores, and short shoots, which grew laterally, produced fronds and were responsible for the vegetative expansion of the plant. Fronds originate from adaxial buds on short shoots, which also carry a large number of dormant buds. See Fig. 4 & 5 for diagrams of fronds and rhizome.

Bracken is perennial - fronds emerge from the ground in spring, usually late May, and continue emerging until the end of the growing season in October when they die back, usually because of frost, forming masses of litter (Watt, 1943). Morphology and density of both fronds and rhizome varies in response to habitat. Fronds in exposed conditions develop a many-layered canopy, whereas fronds in the shade are large and thin with a less upright lamina (Atkinson, 1989).

The expansion of bracken is usually a vegetative spread of the sporophyte. See Fig.6 for the simplified life cycle of bracken. All plants in a contiguous bracken stand can, therefore, be identical clones, or, rather, the entire stand is just one genet. However, like all ferns, bracken can also spread sexually by spore production and then undergo sexual reproduction in the gametophyte generation. In the U.K. spore production is not a regular occurrence. Although some stands sporulate every year, spore production in the U.K. is generally poor (Dyer, 1990). Sexual spread is advantageous in locations where great disturbance events, such as fires, open up new habitats (Dyer, 1990). The extent of sexual reproduction of bracken in Britain, however, has previously been underestimated. Wolf (1990) reported a high gene flow and a high level of genetic variability in British bracken populations. This high genetic variability may account for the genetic basis for bracken's resistance to chemical and biological control.

1.5.2 Ecology

The distribution of bracken in the UK, both latitudinal and altitudinal is largely limited by frost (Watt, 1976). Frond buds and rhizome apices are sensitive to low temperatures, and

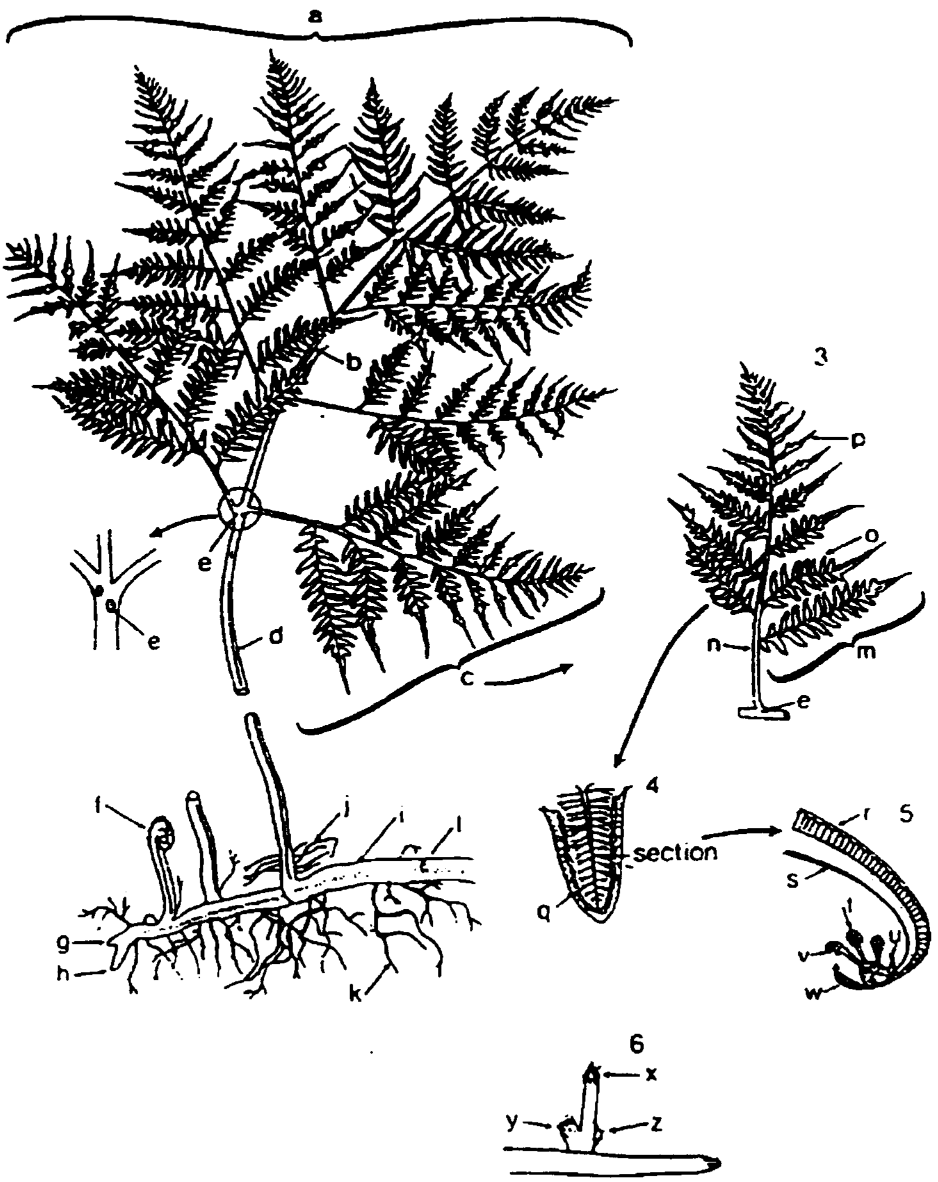


Fig.4 Generalised morphological features of *Pteridium*. **1a** frond lamina (blade); **b** rachis; **c** pinna; **d** stipe; **e** nectary; **f** crozier; **g** leaf primordium on short shoot; **h** shoot apex; **i** lateral line; **j** petiolar roots; **k** root; **l** rhizome; **2e** nectary (minor nectaries are usually present at the bases of the pinnae (**3e**) and some pinnules; **3** pinna, showing **m** pinnule; **n** midrib of pinna; **o** pinnulet; **p** midrib of pinnule; **4** lower surface of pinnule, with **q** coenosorus continuous around margin; **5** section through margin of pinnulet, showing **r** upper surface; **s** lower surface; **t** mature sporangium; **u** indusium; **v** sporangium after discharge of spores; **w** false indusium (margin of segment); **6** rhizome showing **x** frond primordium; **y** abaxial bud; **z** adaxial bud. (From Thompson & Smith, 1990).

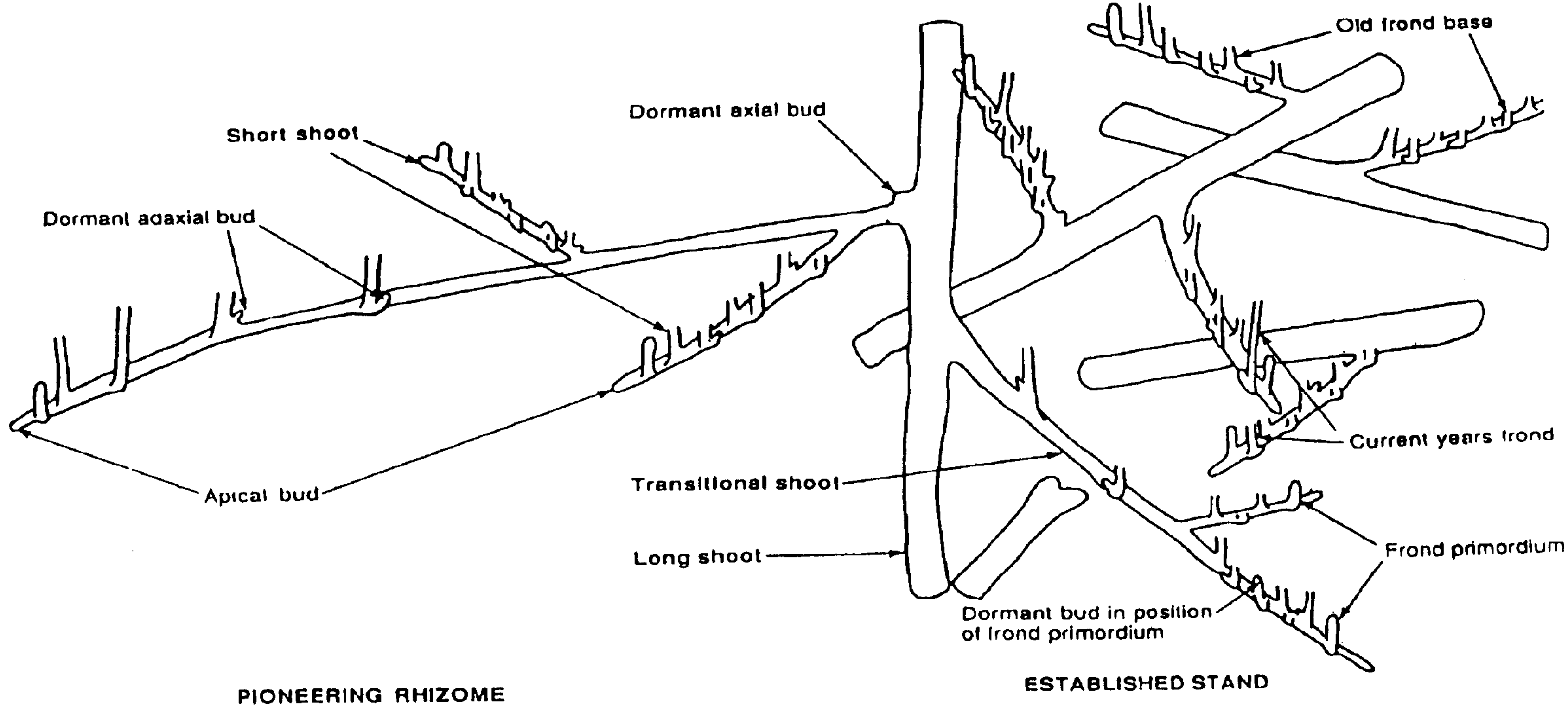


Fig.5 Generalized structure of bracken rhizome. From Whitehead (1993).

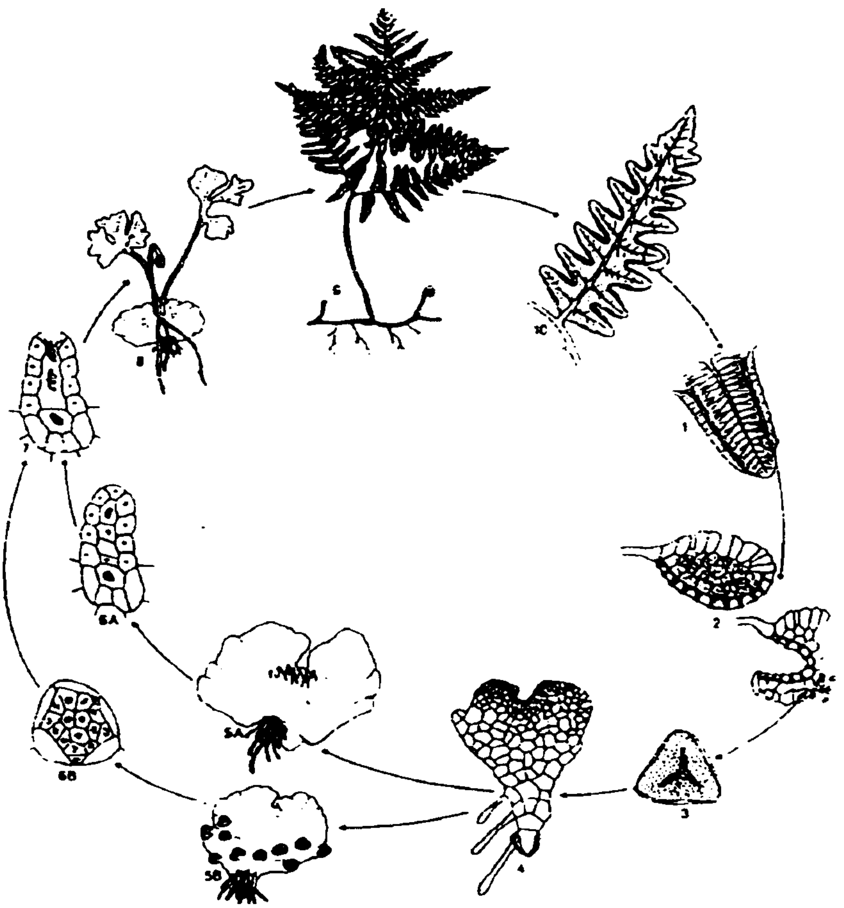


Fig.6 Life cycle of *Pteridium* (generalised). **1** lower surface of a fertile leaf segment (here a pinnulet) showing a single continuous marginal sorus (coenosorus) producing sporangia; **2** a mature sporangium (above) and sporangium after release of spores (below); **3** spore; **4** germinated spore showing developing prothallus with rhizoids; **5** cross fertilisation is normal in *Pteridium*, archegonia and antheridia are not normally formed simultaneously on one prothallus; **5A** larger older prothallus bearing archegonia (**6A**); **5B** smaller, younger prothallus with antheridia (**6B**); 7 spermatozoids released from antheridia move towards mature egg cell; **8** developing sporeling (sporophyte) growing *in situ* on gametophyte. Normally only a single zygote develops from each prothallus. The first leaf of the sporeling is bipinnate; successive leaves are more complexly divided. The prothallus degenerates whilst the growing sporeling withdraws material from it and becomes increasingly independent. The rhizome of the sporeling then develops. The adult sporophyte (**9**) does not usually become fertile (**10**) before 3-4 years of age (Conway, 1957; Dasanayke, 1960). (From Thompson & Smith, 1990).

can be killed by severe winter frosts, especially without the insulation by senescent frond litter. Early and late frosts determine the length of growing season - croziers are damaged by frost, thus delaying the development of frond canopy and reducing frond height (Watt, 1950). This prevents accumulation of litter and compounds the frost problem as litter layer acts as insulation (Ader, 1990). Bracken prefers well-aerated, loamy soils, being absent from bogs, marshes, clay and waterlogged areas (Thompson *et al.*, 1986). The rhizome is sensitive to low - oxygen conditions and high soil moisture prevents rhizome aeration (Poel, 1961).

Bracken is characteristically found on nutrient - deficient acidic soils (pH 4.6-6.8), and is scarce on nutrient-rich soils, but this is thought to be an artefact of history and agriculture rather than due to the biological requirements of bracken (Thompson *et al.*, 1986). Bracken can grow over a wide range of pH and shows increased growth and photosynthesis rates with increased supply of nitrogen and phosphorus (Whitehead *et al.*, 1997). Soil depth is likely to be another important factor, as bracken biomass increases with increased soil depth (Whitehead, 1993).

It is commonly thought that bracken does not support a diverse insect grazing fauna. Lawton (1990b), however, has described 27 U.K. species of insect herbivores on bracken, with a further 11 species feeding on the rhizome. This figure is comparable with other ferns, but most of the bracken species have small populations, and their impact on bracken is considered negligible. This high diversity - low abundance of grazers is attributed to the presence of toxins in bracken and control by predators. Common British bracken herbivores include sawfly caterpillars (*Strongylogaster lineata*), aphids of the genus *Macrosiphum*, mining flies *Chrirosia*, a gall forming moth *Paltodora cytisella*, and gall midge *Dasineura*. It is not known how these insects detoxify the chemicals present in bracken.

In the British uplands, the diversity of plant species found among bracken is generally lower than that of heather moorland (Pakeman & Marrs, 1992b). Shading by the canopy and the deep layer of litter prevent invasion of bracken stands by other plants. Bracken also supports a poorer bird fauna than heather with most rare upland species, such as hen harrier (*Circus cyaneus*) and greenshank (*Tringa nebularia*) not nesting in bracken. Bracken is however, an important habitat for whinchats (*Saxicola rubetra*), nightjars (*Caprimulgus europeaus*) and tree pipits (*Anthus trivialis*) (Radcliff, 1977).

Modern upland vegetation is plagio-climatic and is prevented from succession to woodland by grazing. Bracken invades early successional grasslands, heaths and moors and is capable of persisting for a long time, but its presence is not climatic and is a stage in succession towards woodland. Invasion of trees into bracken appears to be prevented by some of the same factors as for other semi-natural vegetation, such as lack of seed sources and grazing. Bracken vigour, however, may have a threshold above which the tree mortality is too high for successful invasion to occur, and bracken-dominated communities may not undergo a transition towards woodland (Marrs *et al.*, 2000).

1.5.3 Bracken spread in the U.K.

In the United Kingdom bracken was originally a woodland species. Bracken proliferation in the U.K. uplands was made possible by appearance of suitable conditions after deforestation. Loss of shading by trees has not affected bracken, and this fern has been able to maintain high productivity in the newly available exposed habitat, probably due to its ability to restrict its water loss (Pakeman & Marrs, 1992a). High productivity quickly produces stands with closed canopy, which shades out other plants. Presence of bracken creates conditions favourable to bracken, accelerating its spread and consolidating its presence. Accumulation of litter prevents colonisation, and insulates the rhizome from temperature fluctuations. Bracken improves aeration of the soils, this enhancing nutrient cycling (Taylor, 1986). The rhizome acts as a sink for nutrients and carbohydrates and drives the spread through asexual vegetative growth, which can be augmented by sexual reproduction. These qualities, combined with chemical resistance to grazers (Cooper-Driver, 1976) and possible allelopathic effects on its competitors (Gliessman, 1976), have allowed bracken successfully to exploit semi-natural landscapes created by woodland clearance.

Apart from these biological factors several anthropogenic practices have accelerated the encroachment of bracken since deforestation. Bracken used to be harvested regularly on common land for farm animal bedding, thatching and manufacture of potash and soap (Rymer, 1976). Some use of bracken for animal bedding remains, but the rest are no longer practised. A switch from cattle to sheep production in the uplands has further contributed to bracken encroachment, as trampling by cattle was more effective in controlling bracken than trampling by sheep. Increased grazing by sheep removed competition by *Calluna*, a palatable species, from bracken, which is avoided by grazers.

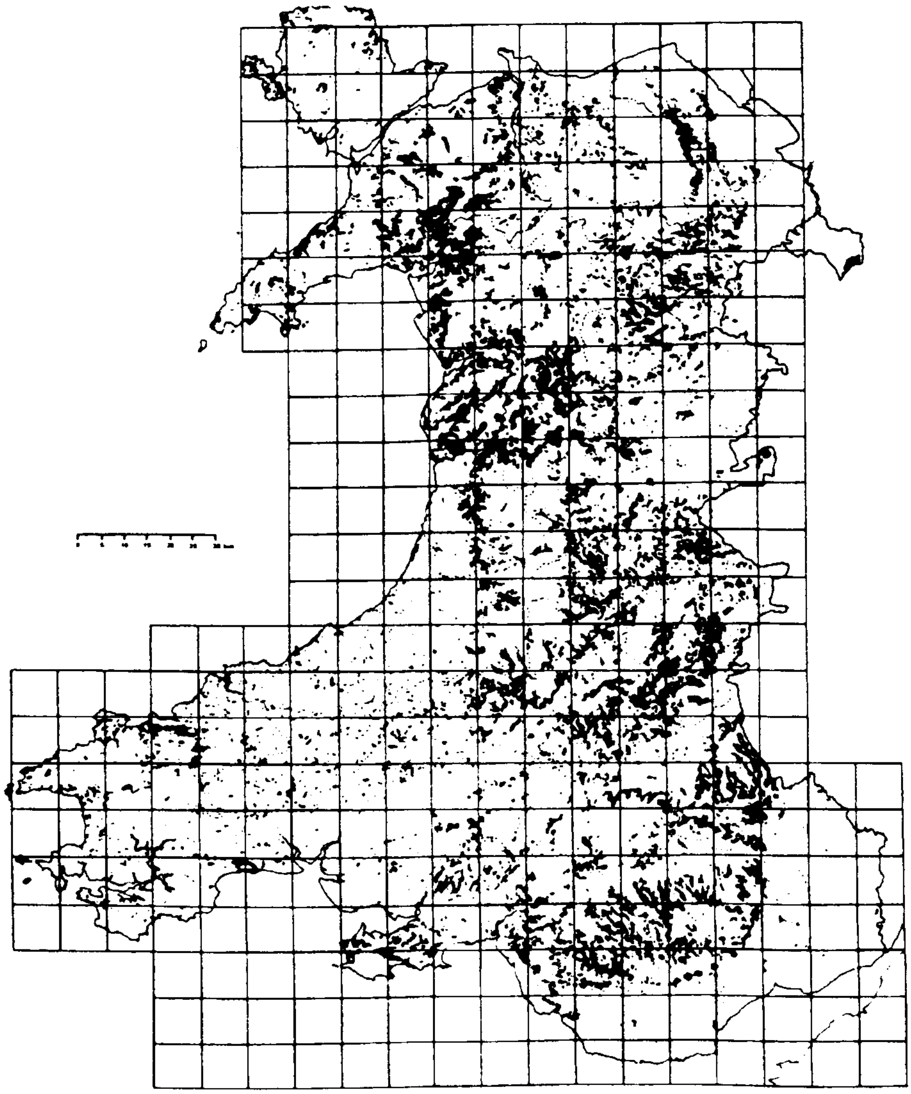


Fig. 7 Distribution of bracken in Wales, based on 1961-1966 vegetation survey. Bracken is the dominant species in all areas shown, and tends to be concentrated in the northwest and the southeast of the principality. Total area under bracken according to this map is 5.7% of total land area of Wales. From Taylor (1986).

The practice of heather burning also played a role. If a burn is carried out at too high a temperature the recovery of heather is delayed and it may take 5-6 years for it to re-establish. Bracken rhizomes, situated deeper in the soil would be protected from the fire and then exploit the area from which the competition has been removed. Burned ground also provides suitable conditions for germination of bracken spores. Controlled burning at normal temperature, however, increases the competitive ability of heather and reduces risk of bracken invasion (Marrs *et al.*, 2000).

Estimates of total land loss to bracken in the U.K. vary among 6720 km^2 (Taylor, 1986), to 3000 km^2 (Lawson *et al.*, 1986) and 2880 km^2 (Bunce *et al.*, 1980). Underestimation of areas with sparse bracken cover, errors in estimating area on steep slopes and extrapolation of local information to the national scale contribute to inaccuracy and variation between estimates. According to Taylor (1986), in Britain, Scotland contains the largest area of bracken, with 2360km^2, accounting for 3% of the total land area, Wales 1241 km^2, or 5.7% of the total land area of the principality (Fig.7), and England 404 km^2 or 0.3% of the land. Similarly to area estimates, those for encroachment rates are also subject to debate. Per annum rates of 0.65% and 3.3% (U.K), were given by Taylor (1986). There is great local variation in encroachment rates, determined by slope and land management. Reliable extrapolation to the national scale is, therefore, extremely difficult.

Bracken biomass in the U.K. is likely to increase with changing climate. A COBRA-X (COntrol of BRAcken eXtended) model (Pakeman & Marrs, 1996) of bracken growth predicts a large increase in bracken biomass in the North of the U.K, especially in the Highlands of central Scotland, but with little change in England and Wales.

1.5.4 Bracken control

Encroachment of bracken on lowland agricultural land is not a problem, as control is done by cultivation. The problem arises in the uplands due to the inaccessibility of hilly terrain, especially as bracken control is a long-term process requiring repeated treatments. Bracken control essentially aims at the eradication of rhizome, although complete eradication is probably not possible (R. Marrs, 2001, personal communication). The rhizome is protected by its underground location, has large biomass, reserves of carbohydrates and nutrients. It is capable of regeneration of fronds from numerous active and dormant buds and resisting control treatments for several years (Lowday & Marrs, 1992). Recovery of bracken a year after chemical treatment is shown on Fig.8.

Fig.8 Recovery of bracken one year after aerial spraying with asulam. Berwyn, North Wales.

Modern methods of bracken control include trampling by stock, cutting/bruising and herbicide treatment (Martin, 1976). Herbicide spraying is the main and the single most effective treatment practised today. Since the 1970s the main chemical used has been methyl 4-aminobenzenesulphonyl carbamate, known as asulam (Thomas *et al.*, 1993). Other previously used herbicides were amitriole (3-amino-1-H-1,2,4-triazole), picloram (4-amino-3,5,6-trichloropicolinic acid) and dicamba (2-methoxy-3,6-dichlorobenzoic acid) (Farnworth & Davies, 1974). These were not well translocated from the foliage to the rhizome, and were non-selective. Glyphosate is another early non-selective herbicide, and is still in limited use today (Petrov & Marrs, 2000).

Asulam is a systemic herbicide, which is readily taken up by the fronds and translocated into the rhizome via the phloem. It reduces the number of fronds produced in the following spring by killing rhizome buds by inhibition of their RNA and protein synthesis (Veerasekaran *et al.*, 1977). Asulam is highly specific and is the only herbicide approved for aerial spraying for bracken control. Reduced number of fronds (up to 95% after the first treatment) leads to a reduction of carbohydrates and nutrients translocated to the rhizome. As treatment continues the rhizome biomass is slowly decreased through respiration and decomposition (Veerasekaran *et.al.*, 1978). Cutting of bracken works along the same principle, denying the rhizome its supply of carbohydrates by destroying the fronds and thus depleting its ability to regenerate. Rhizome carbohydrate reserves are extensive and cutting and spraying must be carried out for several years in order to achieve satisfactory control. Correct timing of cutting and spraying and the combination of these treatments increase the effectiveness of bracken control. Spraying produces the best results when the frond growth is complete and carbohydrates are being translocated to the rhizome, which in the U.K. usually occurs in late July/early August. Optimal time for cutting is therefore from mid-June to late July (Veerasekaran *et.al.*, 1978). When cutting and spraying are used together, the herbicide uptake is more effective. Cutting increases frond density, and therefore the number of uptake points for the herbicide, it also reduces canopy height producing a more even stand, and increases the number of active buds, which are the sink for the herbicide (Lowday & Lakhani, 1987). Reduction in biomass from cutting will also reduce the effective dosage of herbicide (Lowday & Lakhani, 1987).

Marrs *et al.* (1998) showed cutting to be more effective than spraying in reducing rhizome biomass, in the long term. A twice-yearly cut was more effective than cutting once a year, than spraying with asulam without cutting and than spraying followed by cutting. In the short term the combination of cutting and spraying applied once yearly has been shown to be the most effective in reducing frond biomass and number (Le Duc *et al.*, 2000). The long term work of Marrs *et al.*, (1998), however, showed that no treatment succeeded in complete eradication of bracken over 18 years. This example of bracken control attempted to reverse succession and restore heathland/moorland, but it appears that it would be easier and more cost-effective to accelerate natural succession towards woodland instead (Marrs *et al.*, 2000), although this may not always be the desired aim in upland management. Response of bracken to the same treatment varies in different parts of the U.K. This has been attributed to climatic differences and hence different productivity of stands in different regions, but it may also be due to genetic differences between plants in different stands (Le Duc *et al.*, 2000).

Mechanical and chemical methods of bracken control are expensive, and biological control may be a cheaper alternative. The most promising potential control species are South African moths *Panotima spp.* and *Conservula cinisigna* (Lawton, 1990a). These species have different feeding habits from British bracken grazers and would, therefore, exploit a vacant ecological niche. Other possible control agents include a gall forming mite (Lawton, 1990a) and several species of parasitic fungi (Burge *et al.*, 1986). Several obstacles, however, remain before the biological methods can be implemented, such as the breeding of cultures of control organisms, as well as a host of legal and environmental problems associated with an introduction of a new species (Lawton, 1990a).

1.5.5 Biochemistry and toxicity of bracken

Deleterious effects of bracken on human activities, such as bracken poisoning of livestock (Evans, 1982b) have prompted numerous studies on the chemical composition of *Pteridium aquilinum*. Bracken is the only known higher plant causing cancer naturally in animals (Prakash *et al.*, 1996). It is unusual in its ability to produce and retain a large number of toxic secondary metabolites (Cooper-Driver, 1976), which have a wide spectrum of toxic effects. An abundance of information exists on the toxic effects of bracken on animals, primarily mammals, and, to a lesser extent, birds. It is important to maintain the distinction between the toxic effects that occur because of '*natural*' consumption of bracken by animals, and those induced in laboratory conditions by administering compounds extracted from bracken.

1.5.5.1 Diseases caused by bracken

Bracken is normally consumed by domestic herbivores and pigs at times when the usual feed is limited (Evans, 1982b). Young animals are more likely to consume bracken than mature individuals. Eating of bracken by farm livestock is known to cause a number of diseases, which can be classified as either acute or chronic. Little information is available on bracken consumption by wild animals, except for rabbits (Pickworth - Farrow, 1917; Watt, 1981).

One of the acute effects of feeding on bracken by domestic animals is avitaminosis B (Evans *et al.*, 1950). Prolonged consumption of young green fronds by cattle, and, to a lesser extent, sheep, causes a severe depression in bone marrow activity, known as acute haemorrhagic syndrome. It is characterized by hemorrhages on mucous membranes and internal organs and severe leucopenia and thrombocytopenia (Fenwick, 1989). Chronic effects include bovine enzootic haematuria (BEH), typical of ruminants. The symptoms of BEH are haemorrhages in the urinary bladder and tumours of the bladder wall. Carcinoma of the upper digestive tract in cattle (Jarrett *et al.*, 1978), and fibrocarcinomas of the mandible in sheep (McCrea & Head, 1978) also occur after feeding on bracken. Deliberate feeding of bracken or bracken extracts has produced lung, bladder and intestinal tumours in a variety of species (Table 1).

1.5.5.2 Secondary metabolites and their toxicity

The toxicity of several bracken compounds has been confirmed by reproducing naturally-occurring symptoms after administering purified extracts of these compounds to animals. The list of bracken toxins also includes compounds such as cyanogenic glucosides, whose toxicity was measured as a correlation between the extent of grazing on bracken and the amounts of those compounds in bracken.

1.5.5.2.1 Ptaquiloside and pterosins

The precursor of much of the mutagenic and carcinogenic activity of bracken is a norsesquiterpene compound termed ptaquiloside (Fig.9A), which was first isolated by Niwa *et al.* in 1983. It was then discovered to be the active principle behind much of bracken's carcinogeneity (Hirono, 1989).

Table 1. Some of the diseases caused by bracken in animals, either in natural conditions by eating bracken, or upon administration of bracken extracts or compounds.

Symptom	Compound	Animal	Exp/Natural	Reference
Thiamine defficiency	thiaminase type I	horses, sheep	natural	1
Acute haemorrhagic syndrome	ptaquiloside	sheep, pigs, cattle	both	2,3
Retinal atrophy	ptaquiloside	sheep	both	4
Enzootic hematuria	ptaquiloside	cattle	natural	5
Upper digestive tract carcinoma	ptaquiloside	cattle	natural	6
Urinary bladder carcinoma	ptaquiloside	sheep, rats, Guinea pigs	both	7,8, 9,10
Mammary gland papillary carcinoma and adenocarcinoma	ptaquiloside	rats	experimental	10
Intestinal adenocarcinoma	ptaquiloside	Guinea pigs	experimental	11
Ilial carcinoma	ptaquiloside	quail, toads	experimental	5, 12
Colonic carcinoma	ptaquiloside	quail	experimental	5
Caecal adenocarcinoma	ptaquiloside	quail	experimental	5
Kidney haematoma	ptaquiloside	toad	experimental	12
Lymphocyctic leukaemia	ptaquiloside	mice	experimental	13
Pulmonary tumours	ptaquiloside	mice	experimental	13
Incomplete sternebrae fusion	ptaquiloside	mice	experimental	14
Rib anomalies	ptaquiloside	mice	experimental	14
Sterility	shikimic acid	quail	experimental	15
Mutageneic effects	shikimic acid	*Drosophila*	experimental	16

References: 1 Evans *et.al.* (1976), 2 Evans *et al.* (1958), 3 Sunderman (1987), 4 Watson *et al.* (1965), 5 Evans (1968), 6 Jarrett *et al.* (1978), 7 Campo *et al.* (1992), 8 Price & Pamukcu (1968), 9 McCrea *et al.* (1981), 10 Hirono *et al.* (1984), 11Bringuier *et al.* (1995), 12 El-Mofty (1980), 13 Pamukcu *et al.* (1972), 14 Yasuda *et al.* (1974), 15 Prorok (1978), 16 Barber (1969).

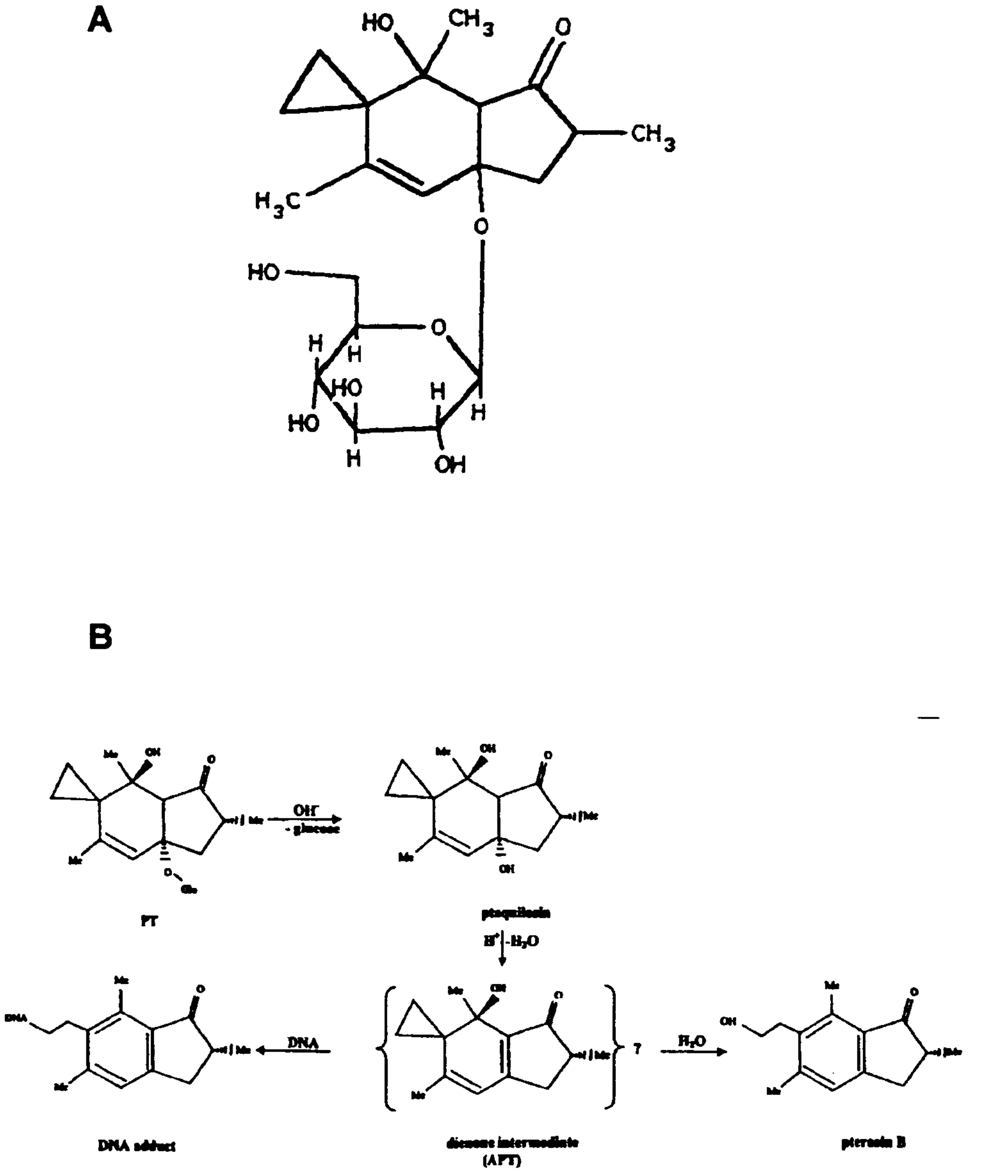


Fig.9 A: molecular structure of ptaquiloside. B: pathway of ptaquiloside reaction with DNA (from Shahin, 1999).

Ptaquiloside is stable at room temperature but decomposes rapidly in aqueous solutions, undergoing light-induced transformation in heated, acidic or basic conditions (Matoba *et al.*, 1987). Under acidic conditions it undergoes aromatisation by elimination of glucose, yielding pterosin B and pterosin O. In aqueous alkaline conditions the transformation is different: an activated form of ptaquiloside (APT) is formed when glucose is eliminated (Shahin *et al.*, 1998). The reactive cyclopropyl group on the APT then binds preferentially to DNA bases (Fig.9B), leading to point mutations. Carcinogenic properties of ptaquiloside are now known to stem from alkylation by APT of a group of oncogenes known as *ras* genes (Prakash et al., 1996). One of the products of these genes is p21 - a GTP-binding protein with a GTP-ase function, which is important in controlling cell regulation. A result of mutation on *ras* genes is a modified p21 with no GTP-ase activity, and the end result is uncontrolled cell growth which is cancer.

Other oncogenes involved in carcinogenic activity of ptaquiloside are *neu* genes - shown to be associated with mammary gland carcinoma in rats, and a tumour supressor gene *p53*, which is involved in several types of human cancer (Shahin *et al.*, 1998). See Fig.10 below for the mechanism of bracken carcinogenesis.

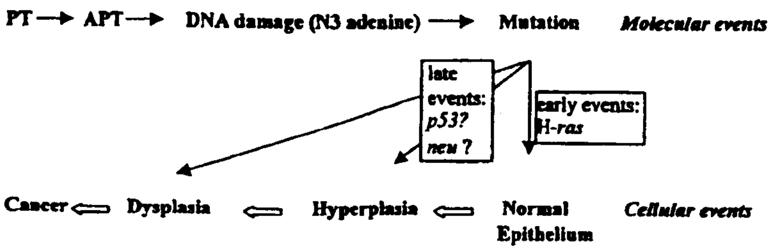


Fig.10 Model of bracken-induced carcinogenesis (from Shahin *et al.*, 1999)

Ptaquiloside is considered to be the carcinogen responsible for high rates of stomach cancer in people living in bracken-infested areas such as North Wales (Galpin *et al.*, 1990), Central America and Andean South America (Alonso-Amelot *et al.*, 1999). Several possible mechanisms of exposure to the toxin have been suggested, including spore inhalation, contamination of water supply and consumption of dairy products from cattle feeding on bracken. The first two pathways remain unproven, but passage of ptaquiloside into milk in animals grazing on bracken was demonstrated by Evans *et al.*, (1972), and further confirmed by Alonso-Amelot *et al.*, (1996, 1999) in Venezuela. Almost 9% of the amount of ptaquiloside ingested by cattle was excreted in milk. The key question here is the intake of bracken by cattle, and hence the amount of ptaquiloside ingested and passed on to humans. Normally bracken is avoided by grazers, but the poorer the pasture the greater the extent of feeding on bracken, and hence the higher the risk of human exposure to significant amounts of carcinogen.

Apart from non-toxic pterosins B and O (Shahin *et al.*, 1999), a number of other pterosins is present in bracken, such as pterosin F (Jones & Firn, 1979) and A (Evans, 1989). The precursor of the latter has not yet been found in bracken, but in *Dennstaedtia scabra* it has been identified as a ptaquiloside - like compound termed dennstoside A (Koyama *et al.*, 1991), which, if present in bracken, may be responsible for some of the mutagenic and carcinogenic activity of *P. aquilinum*. Jones & Firn (1979) judged pterosin F to be partially responsible for the deterrent effects of crude bracken extracts to two insect species.

1.5.5.2.2 Flavonoids, shikimic acid, thiaminase and tannins

Two flavonol glycosides - quercetin and isoquercetin (quercetin - 3 - glycoside), are known carcinogens (Pamukcu *et al.*, 1980). In addition to the established carcinogenic effects to mammals, Nava *et al.* (1987) and Whitehead (1982) have also suggested flavonoids as possible phytotoxins. Todd *et al.* (1971) reported deleterious effects of quercetin on reproduction, growth and survival in a species of greenbug *Schizaphis graminum*. Cooper - Driver (1977), however, has found no inhibition of deer, sheep and locust grazing by flavonoids in bracken, attributing this role to cyanogenic compounds.

Shikimic acid is widely found in nature and had previously been assumed to be non-toxic (Evans, 1982a). Later it was found to be a carcinogen. Evans *et al.* (1950) showed presence in bracken of a thermophyle enzyme system, which destroys thiamine, thus causing avitaminosis B_1. Bracken staggers in horse and sheep are a result of this thiamine deficiency caused by ingestion of bracken and are curable by administration of vitamin B_1 (Evans, 1989).

Bracken metabolises a large quantity of tannins. Tannins procyanidin and prodelphinidin inhibit a number of enzymes by tanning action (Goldstein & Swain, 1965), thus having a broad antibiotic effect, including anti-fungal (Cooper-Driver, 1977). Tannins have been shown to cause a significant reduction in growth of insect larvae in feeding experiments (Carslile & Ellis, 1968; Feeny, 1968). Detrimental effects of tannins on insect growth are attributed to the presence of ortho-hydroxyl groups, also possessed by other known bracken toxins such as caffeic acid and quercetin (Cooper-Driver, 1976).

1.5.5.2.3 Cyanogenic glucosides, terpenoids, phenolic acids and other potential toxins

Cyanogenic glucosides are regarded as the bracken equivalent of alkaloids in higher plants due to the defensive role they both play against herbivory (Jones, 1972). The toxicity of cyanogenic glucosides arises from their chemical instability and subsequent ease with which they are enzymatically hydrolysed to yield HCN (Cooper-Driver, 1976). This occurs when tissue of a cyanogenetic plant is crushed or damaged.

The most widespread physiologically active terpenoid compounds found in ferns are the phytosterols, which are related to ecdysone, an insect-moulting hormone (Herout, 1970). *P. **aquilinum*** contains the highest number of ecdysones for any one plant (Cooper-Driver, 1977). Phytosterols are thought to interfere with growth processes of insect grazers (Robbins *et al.*, 1968).

Phenolic acids have attracted the greatest attention in the search of bracken phytotoxins. Bohm & Tryon (1966) isolated the following cinnamic and benzoic (benzoic derivatives of the cinnamic acids) acids from bracken: *p*-coumaric (p-hydrobenzoic), *o*-coumaric, caffeic (protocatechuic) and ferulic (vanillic). Phenolic acids are known to accumulate in soils. Whitehead (1964) concluded that the amounts of the phenolic acids in soils under bracken were generally lower than those under other plant species. This suggested deleterious influence of bracken on plant growth being due to other phenolics, possibly flavonoids (Whitehead, 1982). Upon their release into the soil phenolic acids may be rapidly detoxified by binding to soil particles, microbial decomposition, or polymerisation to humic acids (Turner & Rice, 1975) before being able to exert toxic effects on the surrounding biota.

1.5.5.2.4 Seasonal chemical variation

Changes in chemical composition of bracken with season have attracted attention as far back as 1949, when Moon & Pal correlated changes in levels of several compounds with palatability of bracken. Cooper-Driver (1977) recorded peaks in the amounts of cyanogenetic glucosides in May/early June with a gradual decline during the growing season. The amount of tannins, however, increased as the season progressed, reaching a peak in late August/early September. Feeding experiments showed that periods of maximum grazing inhibition coincided with the two peaks. Alonso-Amelot *et al.* (1992) and Jones & Firn (1979) revealed parallel changes in concentrations of pterosin F, with peaks in May/early June and subsequent decline, similarly coinciding with feeding inhibition.

The carcinogenic properties of bracken have also been shown to vary with frond development, with younger fronds having greater carcinogenic activity than more mature leaves (Hirono *et al.*, 1990). Ptaquiloside concentrations are highest during the crozier stage and the start of the growing season, and steadily decline with frond maturity (Alonso-Amelot *et al.*, 1992). Work on Southern Hemisphere bracken from New Zealand (*Pteridium esculentum*) has revealed seasonal changes in ptaquiloside concentration (Smith et al., 1990). It appears that total secondary metabolite concentration is highest in young fronds and then steadily declines with maturity.

1.5.5.3 Bracken spore toxicity

Inhalation of bracken spores is another potential mechanism of bracken toxin transfer. Fern spores are known to be toxic and have a deleterious effect on human health. Levels of bracken spores in the air increase drastically during sporulation. Povey *et al.* (1996) recorded an increase in spore count from zero (before sporulation) to 800 per litre, over a bracken stand in North Wales. Povey *et al.*, (1996) discovered formation of DNA adducts in mice administered bracken spores. The exact carcinogen involved, however, is not known.

1.5.5.4 Bracken and the aquatic environment

Terrestrial toxicity of bracken fern is indisputable and is very well documented. Very few attempts, virtually none, have been made to investigate if bracken is toxic to aquatic organisms.

In order for bracken to transport its toxins into water several factors have to coincide. First of all, bracken must be in close proximity to freshwater ecosystems. This is certainly the case in British uplands, where the catchments of small streams, especially in their upper reaches, have extensive bracken cover, which often extends directly into the riparian zone and covers significant areas. The toxins must somehow be leached from the fronds and the rhizome. This study puts forward a hypothesis, initially formulated by Galpin and Smith (1986), that rain leaches out toxins from live fronds and rhizome, as well as from bracken litter, and transports them into streams. In order for this to occur, the toxins must be water-soluble. The main bracken carcinogen - ptaquiloside, is indeed water-soluble. Evans *et al.* (1984) extracted a number of water-soluble carcinogens and mutagens from bracken fronds and rhizome, as well as water-soluble precursors of several toxins (Table 2).

Table 2. Water-soluble toxins isolated from bracken by Evans *et al.* (1984).

Compound	Toxic Status
2,3-butanediol	Mutagen precursor
Proline, 5-oxo-methylester	Possible oncogen
Acetic acid methoxy-ethylester	Precursor of highly toxic methoxyacetic acid
Butanoic acid, 4 methoxy methyester	Increases carcinogenicity of other compounds
Butanedioic acid monomethylester	Potential carcinogen

An important characteristic of chemical compounds required to exert toxic effects in any medium is their stability in that medium. Ptaquiloside is highly unstable and is rapidly broken down to pterosin B and D-glucose under acid conditions, which is important, considering the acidic waters of upland streams in Britain. Factors facilitating ptaquiloside degradation are light and elevation in temperature. It appears unlikely that ptaquiloside would persist long enough in streams to affect stream biota.

Loss of chemical activity of water-soluble bracken extract fractions was also reported by Evans *et al.* (1984), with volatility, molecular instability, oxidation and promoter - initiator separation cited as likely causes. In case of leaching, bracken toxins will have to negotiate exposure to air, passage through soils and finally, water. Behaviour and stability of bracken toxins in each of these media is virtually unknown, and great individual variation between individual toxins is likely. Some indirect evidence regarding soil exists, again from allelopathy studies. Gliessman & Miller (1972), demonstrated an increase in phytotoxicity of soils under bracken canopy after rain, but the validity of the experimental procedure used remains open to doubt. Whitehead (1964) described selective presence of phenolic compounds, to which phytotoxicity of bracken is attributed, in soils supporting bracken swards. Fast - flowing streams are likely to have low bracken toxin residence times, especially as discharge increases after rain. Also, dilution in high flow streams is likely to be high, thus decreasing the likelihood of viable toxic potential.

One study, by Galpin & Smith (1986) has looked directly at the possible presence of bracken toxins in upland streams in North Wales, and deserves close review. The above - national rate of gastric cancer incidence in the county of Gwynedd has led to suggestions of bracken being one of possible sources of carcinogens, and one of the proposed pathways for bracken toxins was contamination of water supply by bracken. In rural North Wales water supplies are localised, usually a being a spring or a stream. Bracken leachate in drinking water was proposed as one of the risk-factors. Galpin and Smith (1986) put forward the following to give weight to the argument that this mechanism of contamination was possible: British uplands are an area of high precipitation, which, combined with sparse vegetation, shollow soils and areas of bare rock, typical of high-altitude, will reduce evotranspiration losses, and produce high levels of run-off, which are theoretically capable of removing toxins from fronds and rhizome. This leachate may then reach the abstraction point of the water supply, a stream, or a reservoir.

Galpin & Smith (1986) then calculated a mean toxin index (MTI) for 21 districts in Gwynedd. MTI was a ratio of total area occupied by bracken in a district to average run-off in that district. No correlation was found between MTI and mortality rates. The conclusion was that bracken contamination of water supply was not a prime cause of stomach cancer in North Wales. Out of 21 districts looked at in the study, however, only five had mountain streams as sources of water, ten districts drew drinking water from lakes, four from springs, one from a reservoir, and one from a borehole. The study, however, did not state how the ground water (springs and boreholes) might become contaminated by bracken. In all probability it does not. Lakes, as potential sources of contaminated water, represent a greatly more diluted source than small streams. It would therefore be more sensible to concentrate on areas where the water supply is derived solely from streams, and discard those where lakes and groundwater are the abstraction points. The effects may only manifest themselves if the abstraction point is relatively high upstream in the mountains, where only the local rural population would be affected, whereas the study included both urban and rural mortality data for each district.

1.6 Hypothesis and aims

The hypothesis of the study was that rain removes water - soluble toxins from bracken, which are then transported into the upland streams, where they exert toxic effects on the biota. The aim of the study was to investigate whether such potential toxic effects did in fact occur on invertebrates and diatoms in Welsh upland streams.

CHAPTER 2

Comparison of physiochemical characteristics of the study streams

2 Introduction

Upland streams are headwaters of rivers and are segments of a greater lotic ecosystem. The stream-river changes as it makes its way from the source to the sea, influenced by changes in the surrounding topography. Unless the entire course of a river is being studied, a study stream/river would amount to the stretch above a selected cut-off point. The streams in this study are the headwaters of the Welsh Dee and the Clwyd. The cut-off points were made high on the moorland, close to the source, resulting in a sample of small, narrow streams. The morphology, chemistry and ecology of such upland streams are shaped and governed by the mountain landscape, climate and geology.

2.1 Physical characteristics of upland streams

Upland moorland streams typically drain small areas of land and are shallow and narrow, containing a small volume of water. This makes them unstable and readily respondent to outside factors. Discharge changes rapidly in response to rainfall, and then returns to previous levels. As uplands are areas of frequent rainfall, the streams have a torrential nature, with frequent periods of stormflow. Seasonal discharge varies depending on the soils and the climatic conditions. Generally, for waterlogged soils, characteristic of moorland, annual hydrographs will show periods of greatest discharge coinciding with the months of maximum precipitation (Hynes, 1970). Coincidence of high flow with precipitation is also strengthened by high run-off due to low evotranspiration, shallow soils and abundance of exposed bedrock in the uplands (Galpin & Smith, 1986).

Because they flow through mountainous terrain, the slope of upland streams is generally steep, so these streams are usually described as 'fast-flowing'. Average flow velocity, however, increases with distance from the source, but upland streams show a greater range of flow velocities over the cross-sectional area, than streams in lower reaches (Hynes, 1970). Extremely low current speeds alternate with sections of foaming, turbulent water. The close proximity of these different channel units provides considerable physical heterogeneity to upland streams. Several schemes have been devised to classify channel reaches according to their hydraulic characteristics and structure. Using Montgomery & Buffington's (1993) scheme, upland streams exhibit mainly cascade and pool-riffle reaches, characteristic of steep alluvial channels. In the cascade reaches water tumbles over large boulders, with smaller sized particles washed out by the action of cascading water. Waterfalls may also be present. Cascade reaches are common in mountain streams and found in their steepest sections. In sections of moderate gradient pool-riffle reaches take over, where shallow fast water sections (riffles) alternate with those with deeper slower- moving water.

Upland streams typically have stony substrata. Large boulders are abundant and there is little organic matter. The pulses of high discharge wash out smaller substratum particles and organic matter downstream (Hynes, 1970). The scouring action of moving stones does not allow the establishment of macrophytes and vegetation is limited to aquatic mosses (*Philonotis fontana*, *Fontinalis antipyretica*, *Hylocomium armorcium*), liverworts (*Solenostoma cordifolium*, *Scapania undulata*, *Nardia compressa*) and periphyton growing on stable boulders (Fitter & Manuel, 1986). The deep, narrow valleys, typical of many upland streams are another product of this scouring action (Hynes, 1970).

Oxygen concentration of the water of upland streams is high, due to high solubility of gases in the cold water, turbulent, well-mixed water flow, and very low decomposition rates of organic matter due to its low input and short residence time in the torrential stream (Allen, 1995).

The temperature of upland streams is largely determined by the ambient air temperature and follows daily and seasonal changes in solar radiation (Smith & Lavis, 1975; Crisp & Howson, 1982). Absolute water temperature is determined by stream size, climatic conditions, local topography and catchment vegetation. Weatherly & Ormerod (1990) reported lower mean daily spring temperatures and higher winter ones for a forested catchment compared with a neighbouring moorland catchment in Wales. Langan *et al.*, (2001) reported mean annual temperature of 8C, with 5.8, 12.5, 7.6 and 2.1 degrees for spring, summer, autumn and winter respectively, for the 1968-1997 period for Girnock Burn, a 30km^2 heather moorland catchment in Scotland. Altitude in the catchment ranged from 250 to 860 m, and stream depth ranged from several centimetres to 0.5m, similar to the streams used in this study.

2.2 Hydrochemistry of Welsh upland streams

Upland streams in Wales are characteristically acidic, low in dissolved organic carbon (DOC) content, with low alkalinity and conductivity. Hydrochemistry of Welsh streams is strongly influenced by the proximity of the sea (Reynolds *et al.*, 1989; Donald *et al.*, 1990), with significant proportion of sea-salt deposited as precipitation (Reynolds *et al.*, 1989). Ionic balance of upland streams is biased towards anions, as is typical of low-strength ionic waters (Reynolds *et al.*, 1984). Water chemistry and solute budgets differ significantly between moorland and grassland streams and those with catchments afforested with conifers. The difference arises from greater atmospheric scavenging by trees and changes that afforestation causes in hydrology of a catchment (Reynolds *et al*, 1989; Stevens *et al.*, 1993).

2.2.1 Individual chemical determinands

2.2.1.1 Nitrate

Nitrate concentrations in moorland streams in the UK are very low compared with their lowland counterparts (Reynolds *et al.*, 1992). Stevens *et al.* (1993) described seasonal changes in nitrate concentrations at Beddgelert, North Wales. The lowest levels (below 0.01 mg/l) occurred in the summer months with an increase of up to 0.4 mg/l in February and March (Stevens *et al.*, 1993). The same seasonal trend had been observed by Reynolds *et al.* (1992) at Plynlimon, Mid-Wales. Low nitrate concentrations are due primarily to low-intensity agriculture and farming in the Welsh uplands, and there are no trends of an increase in nitrate concentrations (Robson & Neal, 1996). The main sources of nitrate are industrial emissions and vehicle exhausts, and rainfall nitrate concentrations display a distinct West - East trend with the highest concentrations occurring in the East, in the vicinity of the urban centres of Liverpool, Manchester and Birmingham. (Donald *et al.*, 1990).

Nitrate forms an example of the influence of riparian vegetation, and conifers in particular, on a budget of a chemical determinand in an upland stream. Concentrations are substantially greater for catchments afforested with conifers, and the seasonal trend is the opposite of that for moorland streams, with concentration maxima occurring in the period May to August (Stevens *et al.*, 1993). Nitrogen supply to upland streams in Wales is mainly of atmospheric origin, delivered by wet, dry, and occult deposition (Reynolds *et al.*, 1994). Atmospheric inputs are greater to the much taller conifers than to moorland grasses, hence there are higher streamflow concentrations in afforested streams. Soils rich in mineralised nitrogen are a terrestrial source of nitrate to streams. Again, vegetation type exerts its influence - conifers increase the amount of nitrogen in underlying soils and the forest floor, whereas moorland grasses *Molinia*, *Festuca* and *Nardus*, whose nitrogen cycle is tighter than that of conifers, (Reynolds *et al.*, 1994) contribute significantly less, thus reducing the amount of soil nitrate available to streamflow.

2.2.1.2 Ions of predominantly marine origin (Na^+, Cl^-, K^+, Mg^{2+})

Reynolds *et al.* (1989) showed Na^+ and Cl^- to be major ionic components of stream water at Plynlimon, mid-Wales, each accounting for 52% and 60% of the cation and the anion sums respectively. Na^+ concentrations ranged from 3.38 mgl^{-1} to 4.65 mgl^{-1} and chloride concentrations from 5.71 mgl^{-1} to 7.73 mgl^{-1} in four streams sampled. Potassium concentrations were less than 4% of those of chloride, in the region of 0.2 mgl^{-1} for all streams. Levels of magnesium were in the 0.07 - 0.09 mgl^{-1} region. The same study, which ran from 1983 to 1985, revealed a seasonal trend for potassium in grassland streams, similar to that for nitrate, with winter maxima and summer minima.

Robson & Neal (1996) conducted a ten-year study of water quality trends at Plynlimon, monitoring rainfall and streamwater concentrations of a number of solutes. Unlike the two-year study of Reynolds *et al.* (1989), where high variation in water chemistry was likely to have obscured seasonal trends, Robson & Neal (1996) showed strong seasonal cycles of Na^+ and Cl^-, with a late-winter peak and late-summer/early autumn minimum. Maximum winter Cl^- concentrations lay between 9 and 12 mgl^{-1} with minima between 6 and 7 mgl^{-1}. The seasonal cycle of sodium was less pronounced, with summer concentrations at around 4 mgl^{-1}, and increases to 5 mgl^{-1} in the winter months. Magnesium showed no seasonal cycle with concentrations around 0.8 mgl^{-1} throughout the year, in close agreement with Reynolds *et al.*, (1989).

The contribution of ions of marine origin to the streamwater chemistry is influenced greatly by weather conditions, such as the quantity of rainfall and the direction of the wind. Reynolds *et al.* 1984, working on the catchment of Afon Cyff (Eastern slopes of Plynlimon, mid-Wales), found that late-autumn/early winter storms brought by

Atlantic frontal systems from the West and south-west, coincided with the highest concentrations of sea-salts in the rainfall. In spring, with low rainfall and high incidence of winds from the East and north-east, the sea-salt concentrations decreased, whereas those of non-marine species, such as nitrate and non-marine Ca^{2+}, increased. Windspeed was also a significant factor: Na^{+}, Mg^{2+} and Cl^{-} loads showed highly significant correlations with strong winds from the seaward directions. Note that these concentrations are for rainfall, not streamwater. Donald *et al.* (1990) and Robson & Neal (1996) have shown that in stream water, concentration peaks for atmospherically - deposited species lag relative to the rainfall inputs by several months.

2.2.1.3 Determinands of mainly non-marine origin

The distinction between species of marine and non-marine origin is not absolute. The same species may be derived from both sources, albeit in different proportions. Note that 'atmospherically deposited' is not equivalent to 'marine'. Industrial emissions contribute significantly for some species to atmospheric deposition.

The concentration of Na^{+} is slightly greater in stream water than in rainfall which suggests an additional secondary terrestrial source (for example a weatherable area within the bedrock or drift of the catchment) of this ion at that location (Reynolds *et al.*, 1989). Calcium, sulphate and magnesium are other examples ions of such dual origin. Calcium levels are low in Welsh moorland streams. The ten-year study of Robson and Neal (1996) showed average concentration of $1 mgl^{-1}$ throughout the year, with no clear seasonal cycle. Calcium in streams is either atmospherically deposited or is transported from underlying bedrock. The latter source is negligible in Wales, but on a local scale small calcite veins can have a pronounced influence (Hornung *et al.*, 1990). This emphasizes the need for detailed geographical and soil data for each catchment, as such veins are not shown on even large-scale maps. The proportion of marine and emission calcium in rainfall varies with season, depending on wind direction and the origin of the frontal systems, (Reynolds *et al.*, 1984). Unlike calcium, silicon in stream water is entirely terrestrial in origin, produced by weathering of silicate in the drift or bedrock of catchments. At Plynlimon the concentrations of silicon were below 1 mgl^{-1} (Reynolds *et al.*, 1989).

2.2.2 Stormflow and baseflow differences

Streamwater chemistry of upland streams is flow-dependent. During storm events the discharge of waters from soils is greatest and the chemistry of streamwater is consistent with that of soil water, enriched in H^+, Al^{3+}, DOC, Fe^{3+}, and manganese. Baseflow waters are derived from groundwater and are enriched in Ca^{2+}, Mg^{2+} and SiO_2 (Reynolds *et al.*, 1989; Soulsby, 1995). Ions of predominantly atmospheric origin (Na^+, Cl^-, $SO^{2-}{}_4$) and those that are strongly influenced by biological activity ($NO^-{}_3$, NH_4, K^+) do not show consistent variation with flow (Reynolds et al., 1989; Soulsby, 1995). A ten-year study, however, has shown that at least for some catchments, iodine and bromide concentrations are slightly higher during storm flows, despite the dependence of these elements on biological processes (Robson & Neal, 1996). Responses of streamflow chemistry to storm events vary within an individual catchment, affected by the magnitude of individual storms, as well as by atmospheric deposition before and after the event. These two factors determine the hydrological pathway of the precipitation into the stream, which in turn determines what chemical species the flow comes in contact with, and transports to the stream (Soulsby, 1995).

2.2.3 Conclusion

Upland streams are small, unstable bodies of water, governed by terrestrial and atmospheric processes, which cause rapid fluctuations in temperature, discharge and levels of chemical determinands. Waters of upland streams are acidic, and have low ionic strength, with concentrations of most determinands much lower than further downstream in the lowlands. Hydrochemistry of upland streams is determined by quantity and quality of precipitation, distance from the sea, the type of soil and bedrock of the catchment, groundwaters of the catchment, type of riparian vegetation, and hydrological processes that dominate the storm run-off. Localized differences in bedrock, riparian vegetation, and topography can result in contrasting hydrochemistry profiles for closely adjacent catchments. In addition to spatial variation between catchments, there also exists high temporal variation in the levels of chemical determinands within each catchment. This emphasizes the importance of long-term studies in order to make valid estimates of average concentrations, seasonal trends and long-term cycles of chemical determinands.

2.3 Selection of study streams

Initially the suitable streams were sought on visits to mountain ranges in North Wales with bracken cover in the catchments. The following ranges were visited: the Carneddau, the Arans, the Glyders, the Rhinogs, the Berwyns and the Clwydin Range. Subsequently the Countryside Council for Wales granted access to maps of the 1984 vegetation survey of upland Wales (Phase I survey). Phase I maps allowed calculation of bracken cover of the catchments already selected from previous visits to the streams, as well as discovery of new locations in North Wales where bracken cover of small stream catchments was substantial, with variation between catchments. The following criteria were used in selection of streams:

a) Location within a group of adjacent streams varying in catchment bracken cover.
b) Proximity of bracken to the stream.
c) Small size of streams.
d) Absence of coniferous trees in the catchments.
e) Suitability for sampling with pond-net.
f) Absence of man-made factors such as dams, wells and possible sources of pollution.

Condition (a) was necessary to reduce variation in non-bracken stream and catchment characteristics that affect the composition of macroinvertebrate communities. Unknown factors may also influence community composition, but neighbouring catchments are more likely to be similar in respect to such factors. Condition (b) was required in order to account for potential instability of bracken toxins in soils. Many catchments, especially the larger ones had large bracken swards several hundred metres away from the stream, but the toxins may be broken down in the soil before entering the stream flow. The reasons behind condition (c) were two-fold. Firstly, the high degree of patchiness in the distribution of benthic macroinvertebrates in running waters makes it difficult to obtain representative estimates of abundance and diversity from larger streams unless the sampling programme is very rigorous with large numbers of replicates taken. Sampling smaller streams reduces the problem. Also, any toxic leachate from riparian bracken would be less diluted in smaller streams, thus increasing the chances of detecting effects. Coniferous plantations (d) in the catchments increase the acidity of stream water. pH and related parameters are considered to be the main factors affecting community composition in small upland streams. It was therefore important to avoid factors that would further increase variation in pH between the streams selected for the survey. Many streams in mountainous terrain are ill suited for sampling (e) due to very steep gradient, presence of a large number of waterfalls and general inaccessibility.

Two groups of streams were finally selected (Fig.11). The first group of 10 streams was located in the Berwyn Mountains. The second group of three streams was in the Clwydian range. Eight of the Berwyn streams (Fig.12) are the headwaters of Afon Ceidiog, a tributary of the Welsh Dee (Afon Dyfrdwy). Two streams - Afon Caletwr and Afon Llynor, are not part of the Afon Ceidiog catchment and are independent tributaries of Afon Dyfrdwy. Two of the Clwydian streams (Fig.13) are the headwaters of Nant y Ne, a tributary of the River Clwyd, The third Clwydian stream (labeled as Reservoir Stream) was on the opposite side of the Clwydian watershed, forming the headwaters of the River Alun, another tributary of Afon Dyfrdwy.

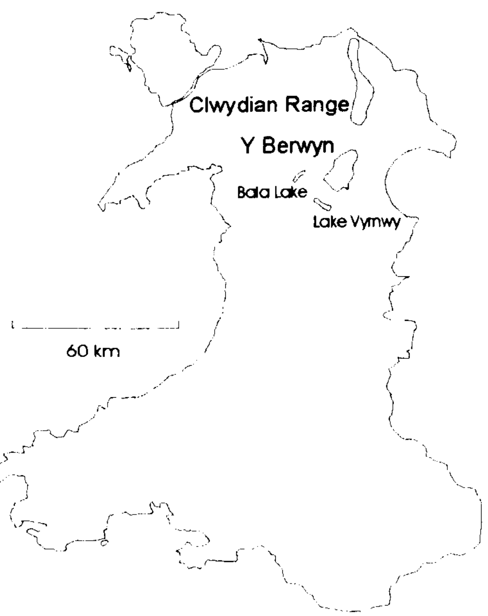


Fig.11 Location of the stream systems used in the study.

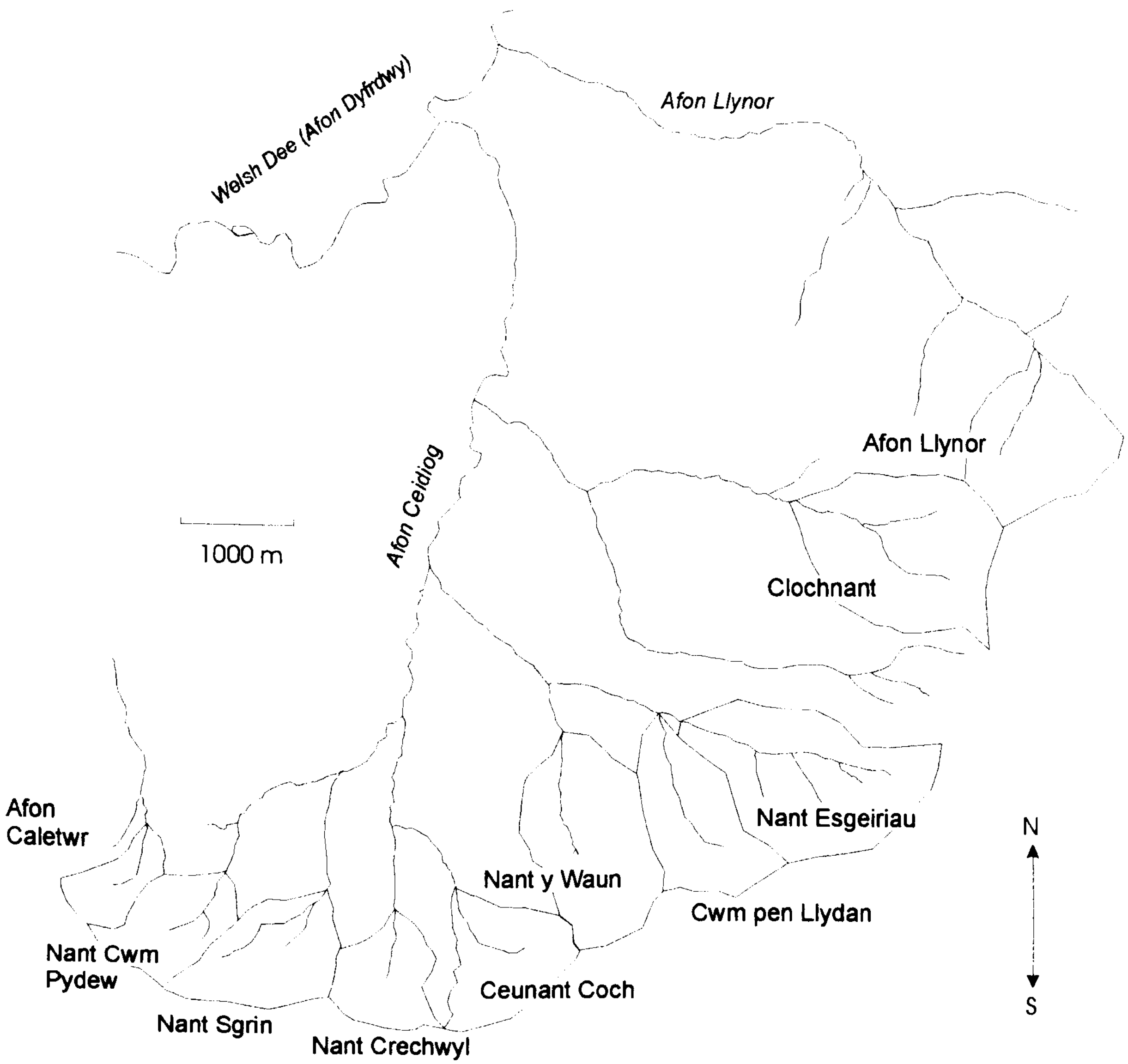


Fig.12 Study streams and their catchments in the Berwyn.

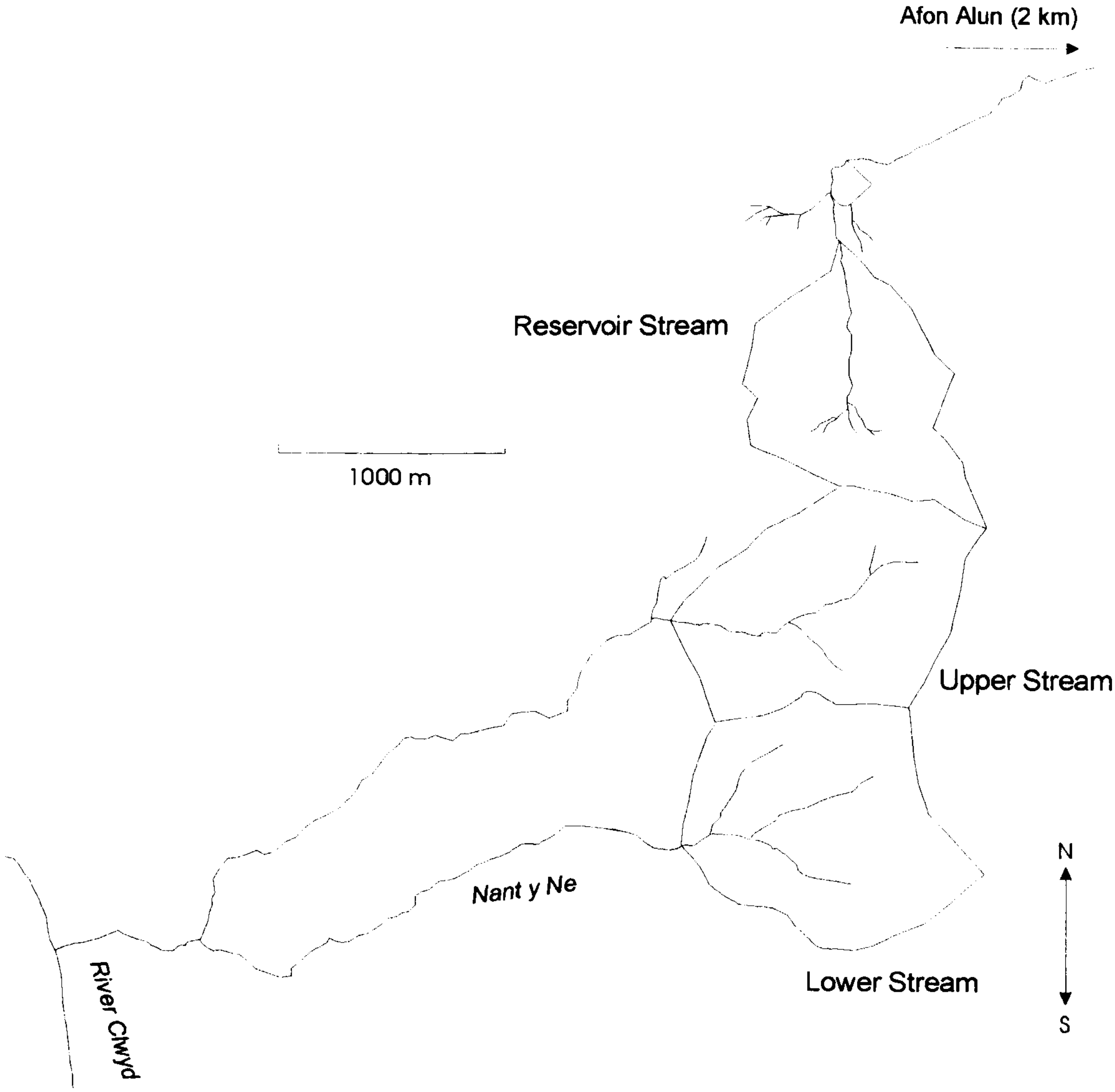


Fig. 13 Study streams and their catchments in the Clwydian Range.

2.4 Introduction to study areas

2.4.1 Y Berwyn

Y Berwyn mountain range (Fig. 14) is situated in North Wales, straddling the counties of Powys, Gwynedd and Clwyd, approximately 50 km inland from the northern coast and 10 km east from Llyn Tegid (Bala Lake). It forms a gentle curve running from Afon Dyfrdwy (Welsh Dee) in the north and merging into the Aran mountain chain in the south-west. The Berwyn is an undulating plateau with gently moulded ridges, and short valleys radiating away from the main ridge. The headwaters of three major Welsh rivers – the Severn, The Welsh Dee and the Dyfi, flow down the Berwyn valleys. The Berwyn is part of Snowdonia National Park, with 16000 hectares of its territory declared as a Site of Special Scientific Interest (SSSI) in 1983, building on the original SSSI of Moel Sych Plateau declared back in 1957 (RSPB & Nature Conservancy Council, 1978).

Main conservation interests in the Berwyn are botanical (Nature Conservancy Council, 1981) and ornithological (RSPB & NCC, 1978). Y Berwyn is regarded as one of the most important areas for upland birds south of the Highlands.

2.4.1.1 Geology

Y Berwyn is part of the upland massif of central Wales syncline which extends from the north-east to the south-west from Llangollen to Cardigan Bay. Two periods of uplifting (Caledonian and Hercynian orogenies) produced the syncline, most of which was then eroded away, and the persisting north-westerly rim is what is now called Berwyn. Y Berwyn consists mainly of Ordovician and Silurian sedimentary rocks - shales, mudstones and sandstones, with some limited areas of volcanic rocks, such as dolerite (Roberts, 1985). The highest peaks are Moel Sych (827m) and Cadair Bronwen (784m). The Berwyn area was heavily glaciated during the last glaciation (Devensian) up to 10 000 years ago (Roberts, 1985). The gently – moulded relief of northern Berwyn is the product of glacial action, as is the thick cover of glacial drift below 500m (RSPB, 1978).

2.4.1.2 History

The Berwyn mountains figure strongly in Welsh history. Two ancient trackways – Ffordd Saeson and Ffordd Gamelin, thought to be between 2000 and 4000 years old,

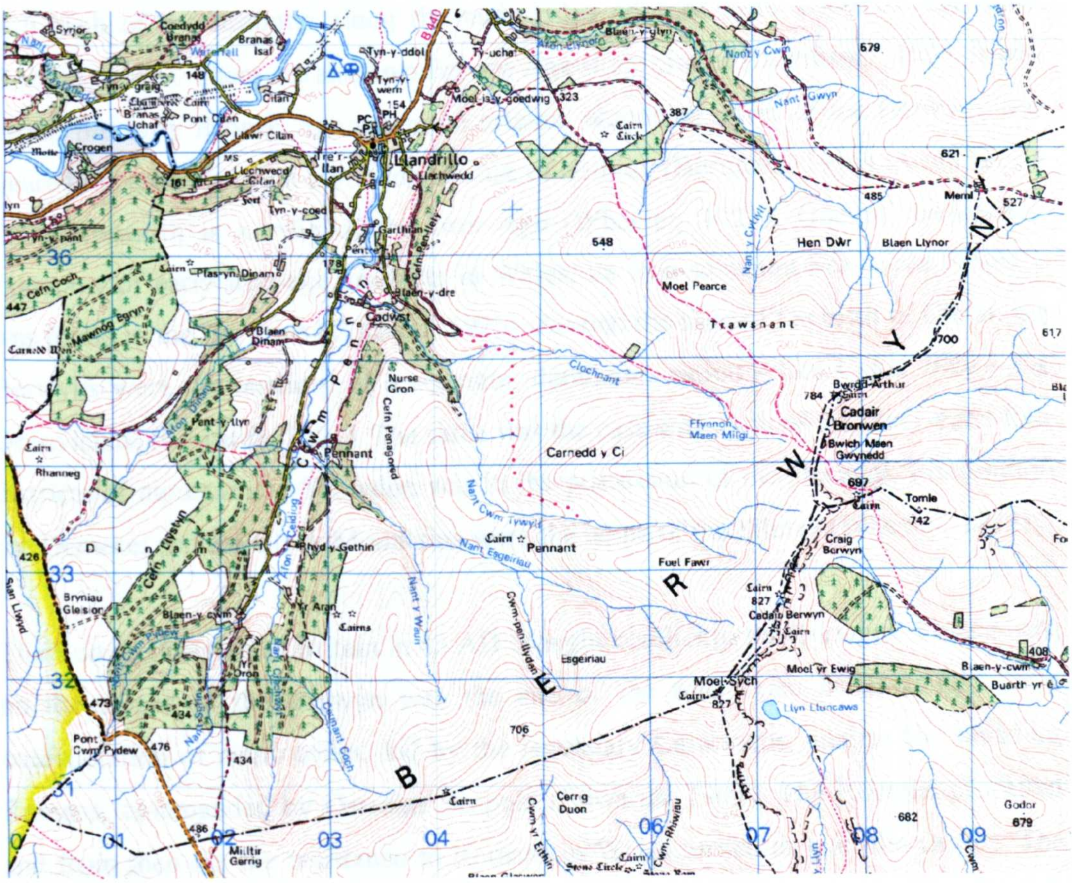


Fig.14 The section of the Berwyn SSSI containing the study streams. Area of each grid is $1km^2$.

dating back to the Neolithic period, are the earliest artefacts of man's activity in the Berwyn (Wheeler, 1925). Bronze age structures are more numerous in the Berwyn, with each of the summits along the main ridge adorned with a Bronze age cairn (Roberts, 1985). There are also two Bronze Age stone circles (Burnham, 1995). It was during the early Bronze Age when the original woodland cover of the Berwyns was cleared for grazing, often involving the use of burning (Burnham, 1995). A hillfort on Craig Rhiwarth is a relic of the Iron Age (Wheeler, 1925). One of the earliest examples of early iron-age artefacts in Wales, an iron-socketed axe (circa 500BC), was found in the Berwyns (Wheeler, 1925). Around the time of the birth of Christ, the Berwyns were the territory of a separate, dominant, nation - like Celtic tribe - the Ordovicians (Edwards, 1901). The same was the case with each of the other main four districts of mountains – Snowdon was in the possession of the Decangi, Plinlimmon the Demeae, and the Black Mountains were the home of the Silures (Wheeler, 1925).

The Roman invasion of Britain in 43 AD brought conflict to the Berwyns. Somewhere on the slopes of the Berwyn was the site of the final open battle fought by a confederation of celtic tribes, led by the legendary Caractacus, against the invading Romans, commanded by Ostorius Scapula (Edwards, 1901). The Romans prevailed and from then on, the resistance to Romans in Wales turned to guerrilla tactics. The Berwyns were again a base for independence activity, this time against the English rule, under the leadership of Owen Glendower, at the beginning of the 15th century (Edwards, 1901). Strong nationalistic and anti - English feeling is still very much part of the mindset of the modern inhabitants of the area (personal observations, 1998-2000). Breaking with the warlike tradition of the Berwyn, a small secluded village of Llanrhaiadr on the eastern slopes of the Berwyn was where the 16th century scholar William Morgan was toiling on the final translation of the Bible into Welsh (Edwards, 1901).

2.4.1.3 Industry

Exploitation of the natural resources of the Berwyn was never on any great scale – a slate industry started in the 17th century, but the inaccessibility of the area limited quarry development (Roberts, 1985). Likewise, metal ore mining was very limited, as was peat cutting (Nature Conservancy Council, 1990). The major industries in the

Berwyn have been farming and managing the moorland for grouse shooting (Roberts, 1985; RSPB & NCC, 1978).

Modern sheep farming practice in the Berwyn has largely done away with open range shepherding, and relies on ring-fenced sheep walks, where sheep are largely left to their own devices, except at gathering times (Roberts, 1985). Increase in sheep farming has led to an increase in converting semi-natural vegetation to improved pasture, which in some parts of the Berwyn penetrates to the altitude of 600m (Nature Conservancy Council, 1981). In the first half of the twentieth century most of the North Berwyn was owned by large estates which had a strong interest in managing heather for grouse shooting (RSPB & NCC, 1978). Most of the Berwyn was the Duke of Edinburgh's shooting estate until 1953, but as most of the estates sold out, the land use changed to sheep farming, leading to a decline in natural vegetation (Roberts, 1985). Some shooting estates are, however, still functioning in the Berwyn (Nature Conservancy Council, 1990). Unlike many other parts of North Wales, such as the Clwydian Range, Snowdon and Cadeir Idris, the Berwyns have little recreational activity (Nature Conservancy Council, 1990).

2.4.1.4 Vegetation

Berwyn contains a wide range of almost intact upland vegetation types that have been lost in other areas to agriculture and afforestation (Nature Conservancy Council, 1990). The hills of the Berwyn contain the most extensive area of upland heath in Wales, as well as one of the best remaining tracts of blanket bog. In addition, several rare plant species are found in the area (RSPB & NCC, 1978; Nature Conservancy Council, 1981, 1990).

Y Berwyn vegetation is a mosaic of various upland vegetation types. The catchments of the study streams, despite their relatively small size, also exhibit several vegetation types, determined by very local variations in soil, slope, aspect, altitude and human involvement (Nature Conservancy Council, 1978). Note that the vegetation described in this section is that of the entire Y Berwyn. The study catchments are a relatively small proportion of this area, and do not comprise all the vegetation types of the greater Berwyn. All figures for area under each vegetation type date back to 1978, as given in a RSPB study of the area.

The waterlogged, peaty plateau of the main ridge is largely a blanket bog with a canopy of heather (*Calluna vulgaris*), cottongrass (*Eriophorum vaginatum*), deergrass (*Trichophorum cespitosum*) and cranberry (*Vaccinium oxycoccus*), which overshadows mats of several *Sphagnum* species. Rare plant species found here include bog rosemary (*Andromeda polifolia*) and cloudberry (*Rubus chamaemorus*). Y Berwyn is the only Welsh location of the latter species (Nature Conservancy Council, 1981). Blanket bogs spill down from the main plateau onto some valley ridges. Total area under blanket bogs in the Berwyns was 6159 hectares (1978, or 32% of semi-natural (excluding agricultural land and coniferous woodland) vegetation.

Dry *Calluna* heaths are found below the blanket bog communities, on steeper, freer draining slopes (Roberts, 1985). These have been managed as grouse moors (Nature Conservancy Council, 1990) and are subjected to regular burning. Total area under heaths in the Berwyn is 3359 ha (18%). Most of the valleys at lower altitudes are under mountain grasslands. The acid, dry, steep slopes are favoured by *Nardus stricta*, *Agrostis* and *Festuca* grasslands, whereas gentler sloping ridges are dominated by purple moor-grass (*Molinia caerulea*). Combined grassland area stands at 5522ha (29%).

Localized water seepage, often close to streams, is frequently associated with soligenous flushes of soft rush (*Juncus effusus*), several species of sedges (*Carex nigra*, *C. rostrata*, and very rare *C. paupercula*), and *Sphagnum recurvum* (Nature Conservancy Council, 1981). Soligenous flushes occupy a relatively small area, 1058 ha, accounting for 5% of semi-natural vegetation. Deciduous woodland is scarce in the Berwyn (total area 72 ha, <0.5%), with two willow species (*Salix cinerea* and *S. aurita*), mountain ash (*Sorbus aucuparia*) and downy birch (*Betula pubescens*), occurring on rock ledges inaccessible to sheep. Extensive plantations (4407 ha) of exotic conifers, such as sitka spruce, were planted after the Second World War (RSPB & NCC, 1978; Roberts, 1985; Nature Conservancy Council, 1981, 1990). Several large forests now exist, such as Cerdiog Forest, Pennant Forest, Penllyn Forest and extensive plantations on the shores of Lake Vyrnwy. There has been large-scale conversion of land to improved grassland, which now covers a total area of 4451hectares.

2.4.1.5 Bracken

Bracken stands are extremely well - established in some catchments with no record of control for the past 50 years (A. Price, SSSI warden – personal communication, 1999). The canopy of these stands is closed, to complete exclusion of other plants. Other catchments, some of which have been sprayed with asulam in the last few years (A. Price – personal communication, 1999) have scattered bracken, where dwarf shrubs and grasses persist under the canopy.

Bracken infestation in the Berwyn appears to be severe. A joint RSPB & Nature Conservancy Council report of 1978 put the total area under bracken at 2454 hectares, or 13% of the total area of semi-natural vegetation in the Berwyn at that time, behind blanket bog and dry heath (50%) and dry acidic grasslands (19%). A later (1990) Nature Conservancy Council report suggested a reduction of the area under bracken by 1600 hectares. The bracken cover in the study area of North Berwyn, however, has not been changed a great deal since the last vegetation survey of the area in 1984 (personal observation, 1999). Some localized spraying has taken place, which destroyed the frond cover, but the stands are recovering because of the failure to follow – up the spraying with necessary eradication measures. Due to inaccessibility of most valleys the future of bracken in the Berwyns is bright.

2.4.2 The Clwydian Range

The Clwydian Range is a narrow chain of hills situated between the valley of the River Clwyd in the west and the river Alun, tributary of the river Dee, in the east (Fig.17). It stretches for 35 km from the town of Prestatyn in the north to the Nant y Garth Pass in the south. The highest peak is Moel Famau at 554 m.

All the information on the Clwydian Range was taken from the latest management strategy handbook for this area (Clwydian Range AONB Joint Advisory Committee, 2000). The Clwydian range was designated as an Area of Outstanding Natural Beauty in 1985, with a total area of 160 km^2. The purpose of AONB status includes protection of fauna, flora, archaeological and architectural features, as well as

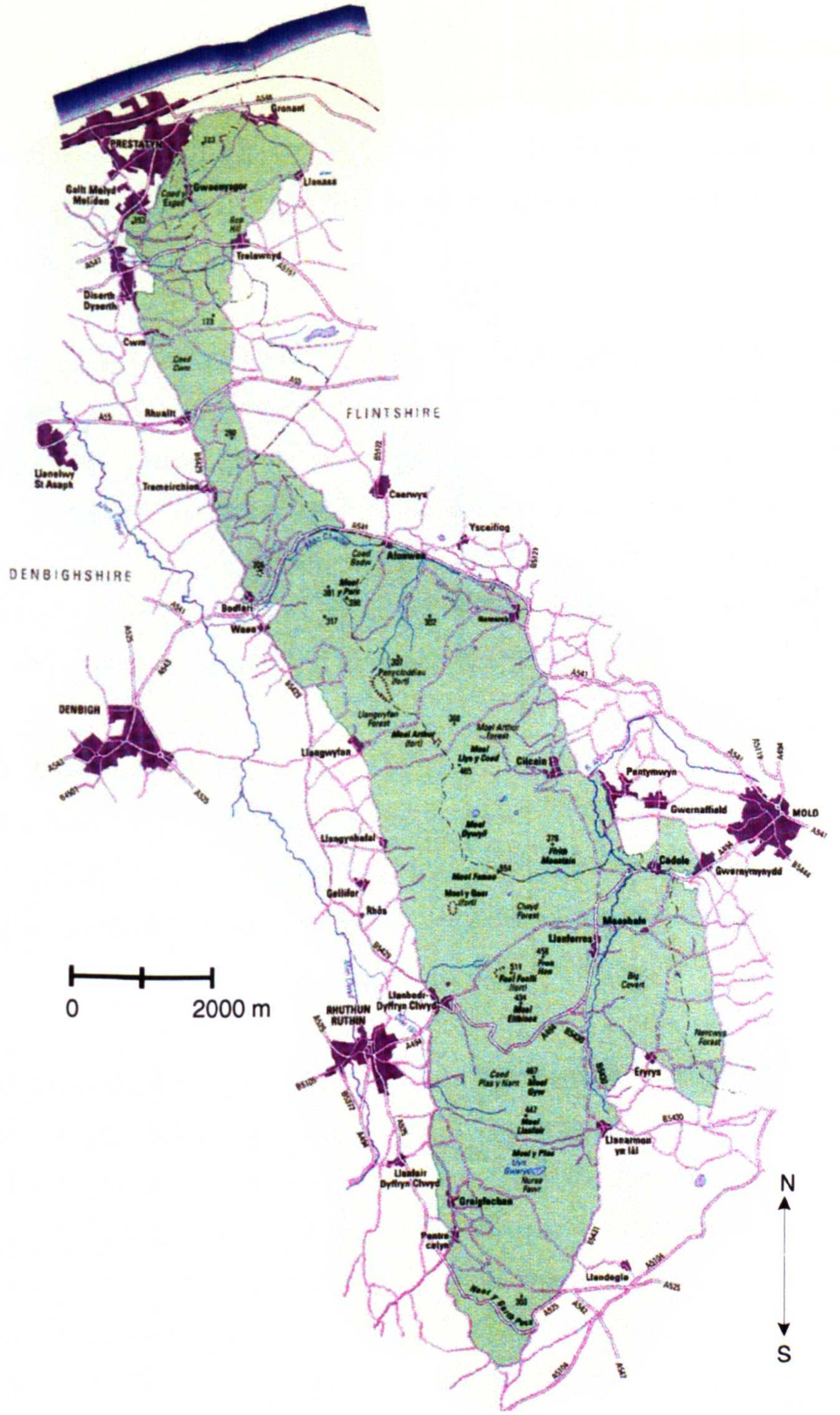


Fig.15 Clwydian Range Area of Outstanding Natural Beauty (AONB).
(From Clwydian Range AONB Joint Advisory Committee, 2000)

promotion of sustainable social and economic development so as to preserve the character and landscape of the area. There are seven SSSIs, four Regionally Important Geological/Geomorphological (RIGS) sites and six Nature Reserves within the AONB. A large part of the common land on moorland ridge has become the Moel Famau Country Park.

The primary conservation interest in the Clwydian Range is calcareous grasslands, some of which have been afforded statutory protection as Sites of Special Scientific Interest, with the largest situated at Loggerheads, Bryn Alyn and on the Prestatyn hillside. A number of rare animal and bird species are found within the AONB such as common pipistrelle bat (*Pipistrellus pipistrellus*), brown hare (*Lepus capensis*), otter (*Lutra lutra*), great crested newt (*Triturus cristatus*), red squirrel (*Sciurus vulgaris*), and pear bodied fritillary butterfly (*Clossiana euphrosyne*).

2.4.2.1 Geology and history

The central spine of the Clwydian hills is composed of Silurian Wenlock shales with bands of sandstone. In the east and the north carboniforous limestone is dominant, with glacial depositions of sand and gravel, especially along the eastern edge and river valleys.

The Clwydian Range contains around a thousand archaeological sites, from prehistoric to the Middle Ages. Early Stone Age sites at Cae Gwyn and Ffynnon Beuno date to approximately 18000BP. These sites are considered an important in understanding of the spread of hunter - gatherers into Britain. Mesolithic tools have been found all over the AONB territory, with Neolithic pottery and stone tools discovered at Gwaenysgor. The Bronze Age is represented by numerous burial monuments. The legacy of the Iron Age is the best-known archaeological feature of the Clwyds - the hillforts. There are six altogether, the largest at Penycloddiau, and the smallest at Moel Arthur. The forts were the controlling centres for territories in the vicinity of the Clwydians, as well as places of tribal gatherings, summer grazings and sites of ritual. Roman archaeological sites have been found in Prestatyn, St Asaph and Ruthin, but there is little evidence of Roman activity in the Clwyds.

Most of the modern villages and farmsteads date from the Dark Ages with many of the churches within the AONB being of medieval origin. During the reign of Edward I the land in the AONB was divided between numerous barons and the modern ownership pattern with the two areas of common land on the crest of the ridge, has origins in this ancient system.

2.4.2.3 Industry

There is a very long history of mineral extraction in the Clwydian Range. Mining has largely been confined to limestone areas in the east of the AONB, where there are some large operational lime-stone quarries. There are also several small silica rock quarries within the AONB. Small-scale quarries are ubiquitous, providing building stone for dry stone walls and buildings.

The main industry in the Range has been farming with most of land, apart from the moorland ridge and the deep valleys, separated by hedgerows into fields, many of which have of improved pasture. Deep valleys and the moorland ridge are used for rough grazing. Recreation is a growing industry in the area. The Clwydian Range is a very popular tourist destination, drawing hundreds of thousands of visitors from North Wales and the North West England. Tourist infrastructure in the AONB is very well developed. There are several recreation areas: Moel Famau and Loggerheads Country Parks, Llangwyfan Nature Area, Moel Findeg and Prestatyn Hillside. Three hundred thousand visitors come each year to the hill top of Moel Famau, and further 200 000 to the Loggerheads Country Park. Offa's Dyke Path National Trail runs the entire spine of the AONB and is walked by thousands of people each year. Other recreational activities practised with the AONB are fell running, climbing and hang gliding.

2.4.2.4 Vegetation

The range can be divided into several distinct zones, which vary in vegetation composition. The main ridge is under continuous dry *Calluna* heath, with some billbery (*Vaccinium myrtillus*), gorse (*Ulex gallii*), and patches of *Festuca ovina* and *Agrostis* grasslands below. The large Clwyd Forest and numerous smaller coniferous plantations are the legacy of the extensive afforestation carried out after the Second World War.

The steep hillslopes and valleys on the western side have dry *Calluna* heath in the upper reaches which is then replaced by dense tall bracken and improved grassland. Gorse is also abundant, especially in lower reaches of the valleys together with a scattering of ash, sycamore and oak trees. Some of the smaller valleys harbour fragments of ancient woodlands. The eastern slopes have been more influenced by agricultural improvement with greater areas of improved grassland.

The limestone areas in the east of the AONB at Graig Fawr and Dyserth Mountain are a mixture of unimproved calcareous grassland with cowslips and orchids, extensive broadleaved woodlands in the valleys and farmland with improved limestone grassland.The gentle coastal slopes of the Range south east of Prestatyn, are covered by agricultural fields defined by well maintained hedgerows. Steeper slopes harbour abundant broadleaved woodland.

2. 5 Methodology - physiochemical variables of study streams

2.5.1 Stream habitat classification

On each study stream a 100m section was measured and marked with bamboo poles. Within this section habitats were classified visually using a modified Montgomery & Buffington (1993) scheme. The following habitat types were recognised: riffles, runs, pools, cascades and sheets (Fig.16). Riffles were fast-flowing, turbulent sections with white water, but without large boulders and bedrock. Pools were deeper habitats, with slow-flowing water and accumulation of fine substratum particles. Runs were intermediate in current speed between pools and riffles, without turbulence and with intermediate substratum size. Cascades had fast, foaming water with large boulders, steep gradient, waterfalls and plunge pools. These plunge pools were included as part of cascade units as they were formed by bedrock scouring and did not accumulate fine substratum particles. Sheets were sections were water ran over bedrock. Sections of the stream where the channel ran underground were classified as 'cover'. Each habitat unit was marked by colour-coded bamboo poles. Classification was carried out in May 1999 during baseflow conditions.

Measurements of width and depth were taken along the entire 100m sampling section of the stream at the intervals of 1m, except in cascades and sheets due to their

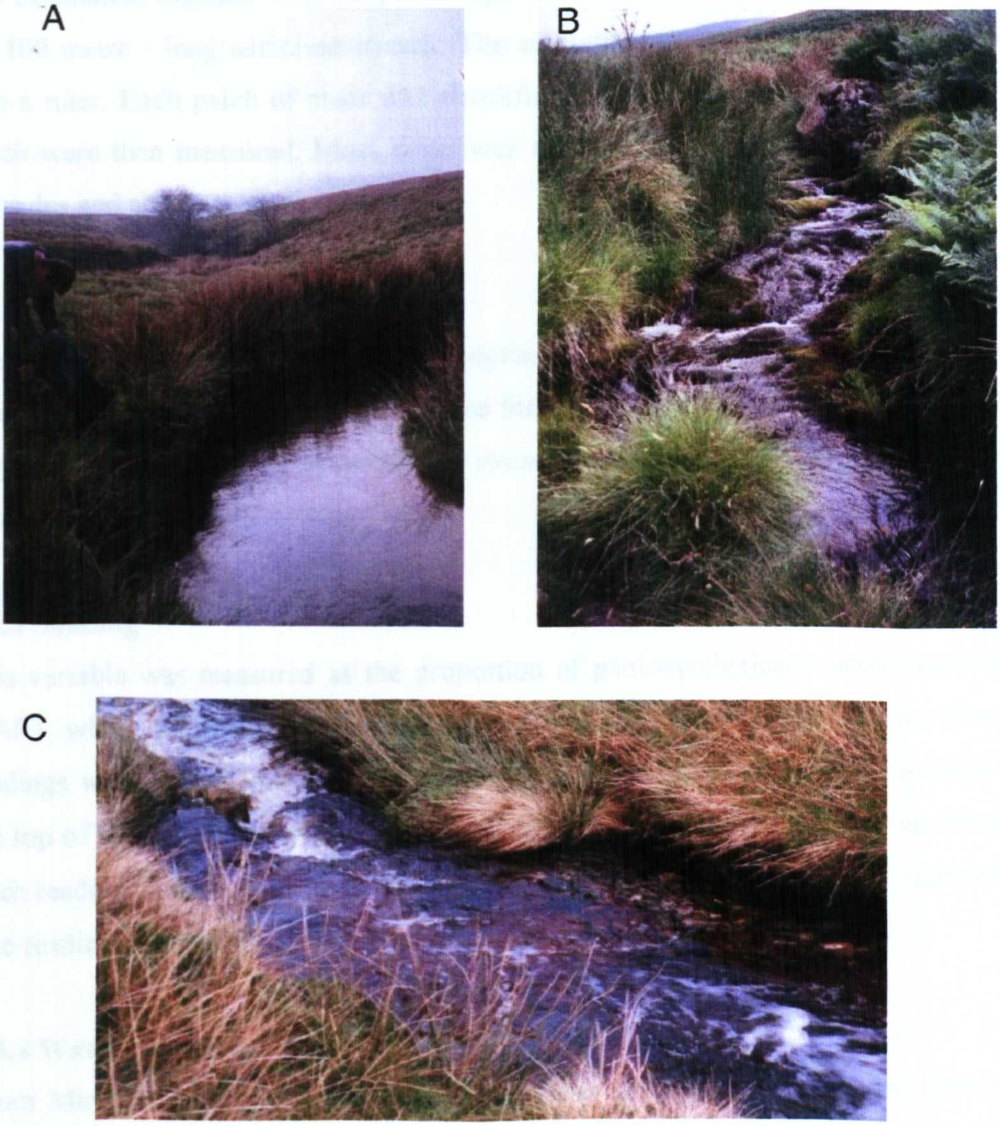


Fig.16 Stream habitat types recognised in this study: A - pool, B - cascade, C - riffle.

inaccessibility. The area of each habitat unit was then calculated by multiplying the mean width of that habitat by its length. The total area of riffles, pools and runs was then calculated, together with the percentage of each habitat type of the total area of the 100 metre - long sampling stretch. The area under aquatic moss was measured with a ruler. Each patch of moss was simplified visually to a rectangle, the sides of which were than measured. Moss cover was measured for each riffle run and pool. Cascades and sheets were not sampled.

2.5.2 Altitude and catchment area

The altitudes of the sampling sites (the beginning of the 100m sampling stretch) was determined from 1:50 000 scale Ordnance Survey maps. Catchment area and stream length was determined by scanning the maps and then using a shareware graphics package.

2.5.3 Shading

This variable was measured as the proportion of photosynthetically active radiation (PAR), which penetrated to the water surface in the centre of the stream. Eleven readings were taken (Macam Q101 Quantum photometer) at an exposed position at the top of the valley and then eleven readings at one - metre intervals at the stream. Each reading at the stream surface was immediately followed by one at the valley top. The readings were taken in cloudless conditions in August 2000.

2.5.4 Water chemistry

From May 1999 until April 2000 monthly water samples were collected from the study streams. Conductivity (Jenway 4010 meter) and pH (Hanna HI 9025 microcomputer meter) were determined immediately on the return. Alkalinity, Cl^- (Mackereth et. al., 1978, titration method), nitrate nitrogen (Mackereth *et al.*, 1978), ammonia nitrogen (Chaney & Morbach, 1962), total suspended solids (TSS) (Mackereth et. al., 1978), Fe^{2+}, Ca^{2+}, Mg^{2+} and Na^+ (atomic absorption flame spectrophotometer Varian AA-1275, unfiltered sample) were analysed the following day. Water in the study streams was tested for the main bracken carcinogen ptaquiloside on one occasion. The 25 ml water samples were collected from the streams in September 2000 after a period of heavy rainfall, as the study hypothesis states that the toxins are removed from bracken by rain. The samples were then analysed at Menai Organics Ltd laboratory at Bangor, Gwynedd. The samples were tested by UV spectroscopy in order to detect ptaquiloside and/or its breakdown product pterosin B. Thin layer chromatography was also used to test for pterosins. These methods of analysis followed the technique of Saito *et al.* (1989).

2.5.5 Bracken

Twenty - four 0.5 m^2 quadrats were used for each catchment, the number of live fronds in the quadrat was counted and the height of each frond measured. The quadrats were arranged in four transects with six quadrats in each. Each transect began at the top of the valley, on the top edge of the bracken stand. I then descended down the valley side, visually estimating the location of the next quadrat so that all six quadrats evenly covered the entire length of the slope. At each quadrat location the quadrat was tossed vertically into the air and the location of landing was the sampling point at that level of the transect. If bracken stands covered both sides of the valley, two parallel transects were taken on each side. In catchments where the stand on one side of the valley was much greater than on the other (Nant y Waun), three transects were taken on the larger stand, and one on the smaller one. Completely randomised sampling was impractical, as impossibility of navigation and orienteering in large swards of high dense bracken, often on steep slopes, made location of chosen random sampling points almost impossible. Bracken density and frond length were measured once, in August 2000.

Area under bracken in each catchment was calculated in the field for the streams which did not agree with 1984 Phase I vegetation survey maps (Clochnant, Nant Esgeiriau, Nant Cwm Llydan) and where bracken cover was too small to be included on these maps (Afon Caletwr). A rope with 1 metre markers was used to calculate the area of the stands. Where bracken cover agreed with Phase I, the maps of the catchments were digitised and the areas under bracken measured using shareware software. The total measure of bracken for each catchment was calculated as the product of mean frond height in the catchment, mean frond density, and the area under bracken in the catchment. It would have been preferable to calculate total frond biomass for each catchment, but biomass can vary significantly for fronds of the same height (M. Le Duc, personal communication, 1999).

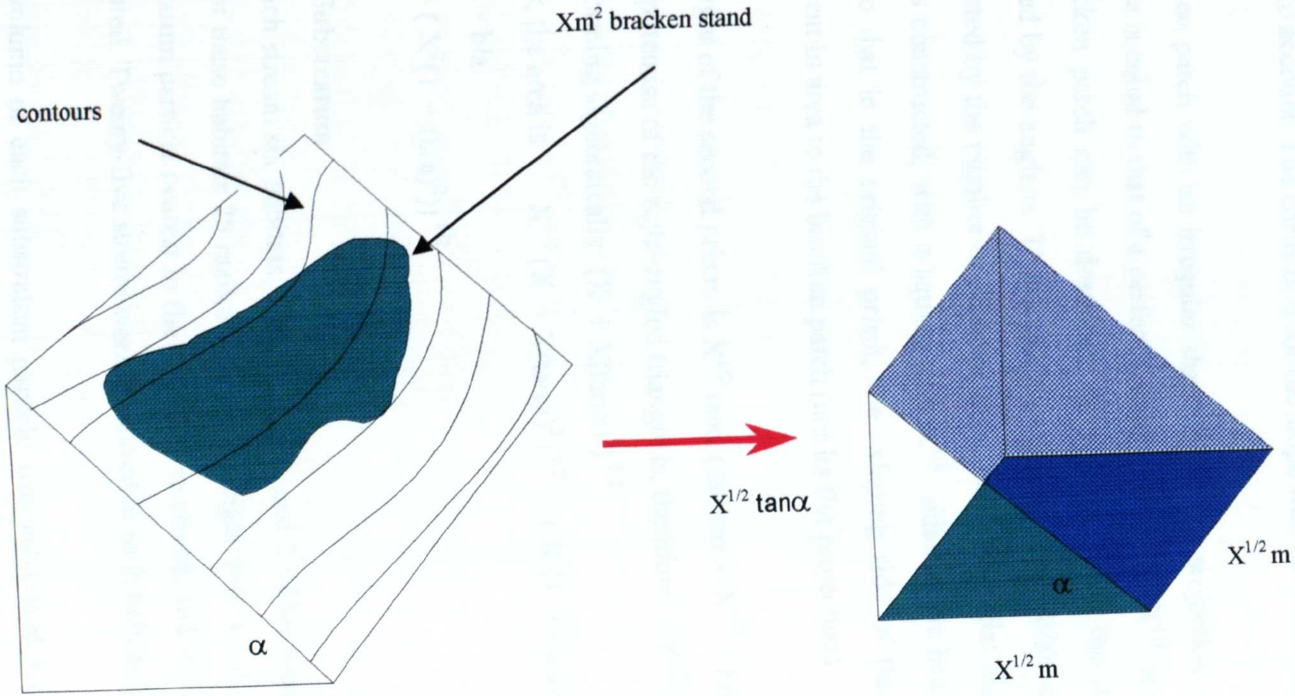


Fig.17 Calculation of the area of a bracken patch situated on a slope.

2.5.5.1 Correction for slope

Bracken stands were often situated on steep valleys, and measuring flat projections of their areas on the map leads to underestimation of the actual areas, as the slope is not taken into account. The correction for the slope was carried out as follows (Fig.17).

A bracken patch with an irregular shape has a flat projection with an area of Xm^2. This area is equal to that of a perfectly square patch with $X^{1/2}$ m sides. The valley with the bracken patch can be described as a prism where the slope of the valley is expressed by the angle α. This angle is calculated as tan(b/a), where a is the distance represented by the number of contour lines contained in flat distance b. If the second prism is constructed, with a square with $X^{1/2}$ sides as the base, and the angle alpha equal to that in the original prism, the sloping side of the new prism (blue) is equivalent in area to the bracken patch (not its flat projection).

The height of the second prism is $X^{1/2} \tan\alpha$ (as $\tan\alpha = X^{1/2}$ / height).

The hypotenuse of the right-angled triangle is, therefore: $((X^{1/2})^2 + (X^{1/2} \tan\alpha)^2)^{1/2}$

Transforming algebraically: $(X + X(\tan\alpha)^2)^{1/2}$

Finally, the area is: $X^{1/2} (X + X(\tan\alpha)^2)^{1/2} = (X^2(1 + (\tan\alpha)^2))^{1/2}$

As tan = b/a:

Area = $(X^2(1 + (b/a)^2))^{1/2}$

2.5.6 Substratum

For each stream six habitats: 2 runs, 2 pools and 2 riffles were selected randomly. In each of these habitats 25 markers (a metal weight with a red float) were thrown. The substratum particle nearest to the float was removed, and its length, width and height measured. Twenty-five stones were measured in each habitat.

The volume of each substratum particle was calculated using the formula for the volume (V) of an ellipsoid, where a, b, and c are the length, width and height respectively of a substratum particle.

$V = 4/3\pi abc.$

Taking a mean value for particle volume for the entire stream, based on equal number of stones from each habitat type assumes that the habitats (riffles, runs and pools) occur in the same proportion in each stream. Some streams however, had their habitat structure heavily biased towards certain habitat types. For example there were very few pools in Ceunant Coch and the Upper Stream. According to this sampling method, pools would be greatly overrepresented and would push the stream mean down due to their low particle size. To avoid this, the means from each habitat type were weighed according to the abundance of that habitat in the stream. A riffle : run : pool area ratio (A : B : C) was calculated. The mean substratum stream values were then calculated as follows:

$$\text{Stream mean} = \frac{A(\text{Riffle mean}) + B(\text{Run mean}) + C(\text{Pool mean})}{3}$$

2.5.7 Periphyton chlorophyll a

Periphyton was sampled once in May, July and September 1999. During each season three riffles were chosen at random for each stream. A float was thrown into each riffle and an embedded stone closest to the float was removed. An area of 25 cm^2 was scrubbed clear of biofilm with a piece of velcro. The biofilm was then transferred into a sampling bottle. In the laboratory the sample was diluted to 300 ml. One hundred millilitres were filtered through 4.5cm diameter Whatman glass-fibre filters (GF/C), and frozen for chlorophyll analysis. Two 100 ml volumes were preserved in 5% Lugol's solution for diatom count. The chlorophyll analysis was performed as follows, the method taken from Richards & Thompson (1952). The filter paper was ground up in the mortar with a pestle after addition of 1ml of a 5% magnesium carbonate solution and a pinch of sand. The contents were then transferred to a 15ml plastic centrifuge tube and topped up to 10 ml with acetone. The tube was then sealed with a bung. The tubes were stored in a refrigerator at 4C° in the dark for 24 hours. Then the contents of the tubes were centrifuged at 4500 r.p.m. for 10 minutes. The supernatant was transferred into 1 cm glass cells and the absorbance measured at 750nm (to correct for fine colloidal matter in the samples) and then at 663nm against an acetone blank. The amount of chlorophyll per 25 cm^2 was calculated using the formula (Talling & Driver, 1961):

Chlorophyll (μg) = 11 x (absorbance (663) - absorbance (750)) x V

11 - a standard value based on the specific absorbance of chlorophyll a.
V - volume of acetone used

2.6 Results

2.6.1 Altitude, stream length, catchment area, moss

The altitude (Fig.18A, on the graphs the westernmost Berwyn streams are first, then Clwydian streams in order in which sampled) of the Berwyn streams varied from 340 m above sea level (Nant Crechwyl), to 470 m (Cwm Pen Llydan and Nant Esgeiriau). The Clwydian streams were situated at lower altitude: 200 m for both Lower and Upper streams, and 290 m for Reservoir stream. The length of the study streams (Fig.18C) was around 1100 m, with catchment sizes approximating 1km^2. The two largest streams were in the Berwyns: Nant Esgeiriau (2176 m) and Clochnant (1776 m). These streams also had the largest catchments (Fig.18B) of 2.02 km^2 and 2.06 km^2 respectively. The shortest streams were Nant Crechwyl (664 m; 0.84 km^2), Nant Cwm Pydew (776 m; 0.7 km^2) and the Lower Stream (703 m; 0.65 km^2). Aquatic moss (Fig.18D) covered between 1% (Afon Llynor) and 14.6% (Clochnant) of stream substratum in the Berwyns. In the Clwyds, the Upper stream had the greatest moss cover of all 13 streams with 20.5%.

2.6.2 Habitat structure, width and depth

No single habitat unit was dominant in the streams (Fig.19). In the ten Berwyn streams four had pools as the main habitat type (Nant Sgrin, CPL, Clochnant and Afon Llynor), three had runs (Nant Crechwyl, NYW and Nant Esgeiriau) and three riffles (Afon Caletwr, NCP and Ceunant Coch). In the Clwyds the riffles were the main habitat units in all three streams, with pools being very rare in Upper (2.9%) and Lower (1.1%) streams. Reservoir stream had a much larger proportion of pools at 25%.

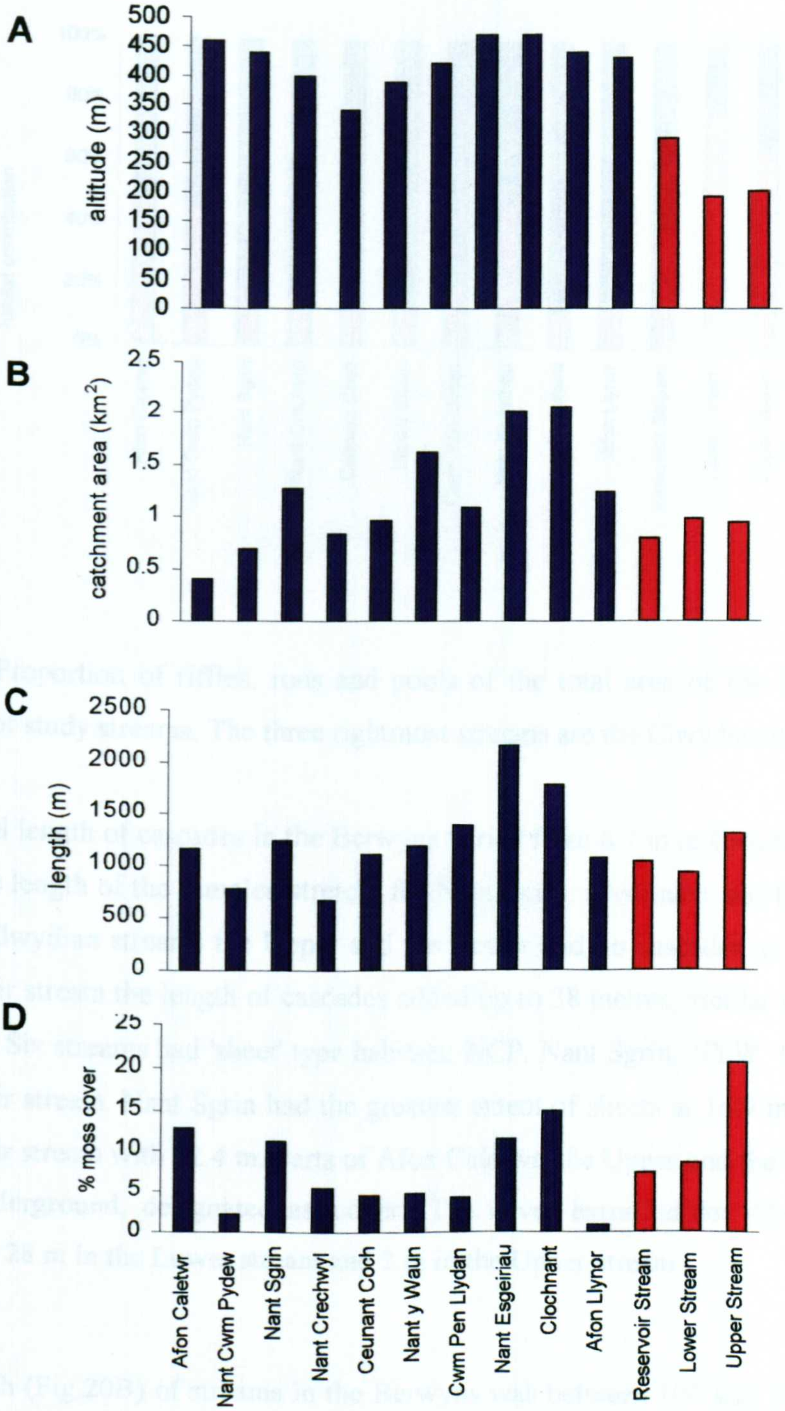


Fig. 18 Altitude (A), catchment area (B), length (C) and percentage moss cover (D) of the study streams. Clwydian streams in red.

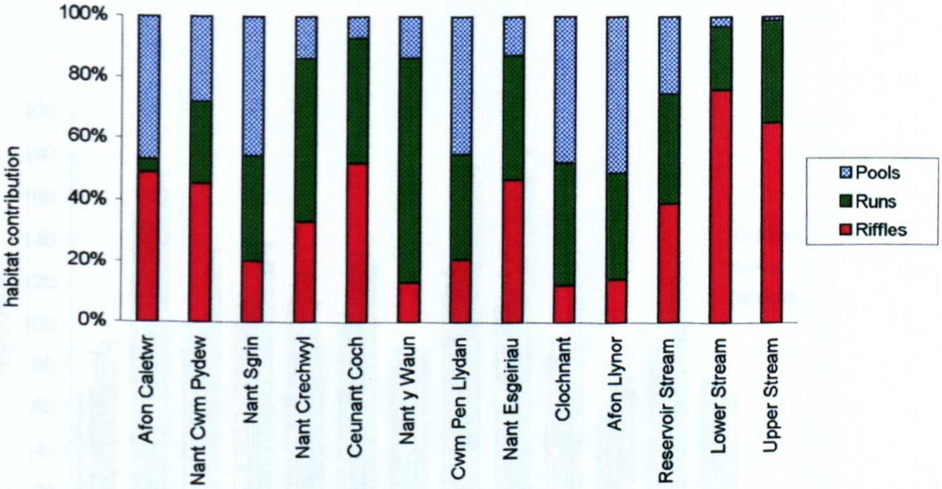


Fig.19 Proportion of riffles, runs and pools of the total area of the 100m sampled stretch of study streams. The three rightmost streams are the Clwydian ones.

The total length of cascades in the Berwyns varied from 6.7 m in CPL to almost 50 m (half the length of the sampled stretch) for Nant Sgrin, Clochnant and Ceunant Coch. In the Clwydian streams the Upper and the Lower had no cascades at all, but in the Reservoir stream the length of cascades added up to 38 metres, similar to the Berwyn streams. Six streams had 'sheet' type habitats: NCP, Nant Sgrin, NYW, Clochnant and Reservoir stream. Nant Sgrin had the greatest extent of sheets at 14.9 m, followed by Reservoir stream with 12.4 m. Parts of Afon Caletwr, the Upper and the Lower stream flow underground, designated as 'cover'. The cover extended for 43.6 m in Afon Caletwr, 28 m in the Lower stream and 2 m in the Upper stream.

The width (Fig.20B) of streams in the Berwyns was between 100 and 155 cm except for the two much narrower streams, Afon Caletwr (73 cm ±26.5SD) and Cwn Pen Llydan (79.4 cm ±30.2SD). The Clwyd streams were narrower at 66.4cm ±29.9SD (Reservoir), 84.4 cm±33SD (Lower), 74 cm±16.2SD (Upper). The differences in width were significant (ANOVA, d.f. = 12, F = 41.34, $p<0.01$), driven primarily by the differences between the Clwydian and the Berwyn streams (post-hoc HSD unequal n test $\alpha=0.05$). Berwyn streams were also deeper (Fig.21B), ranging from

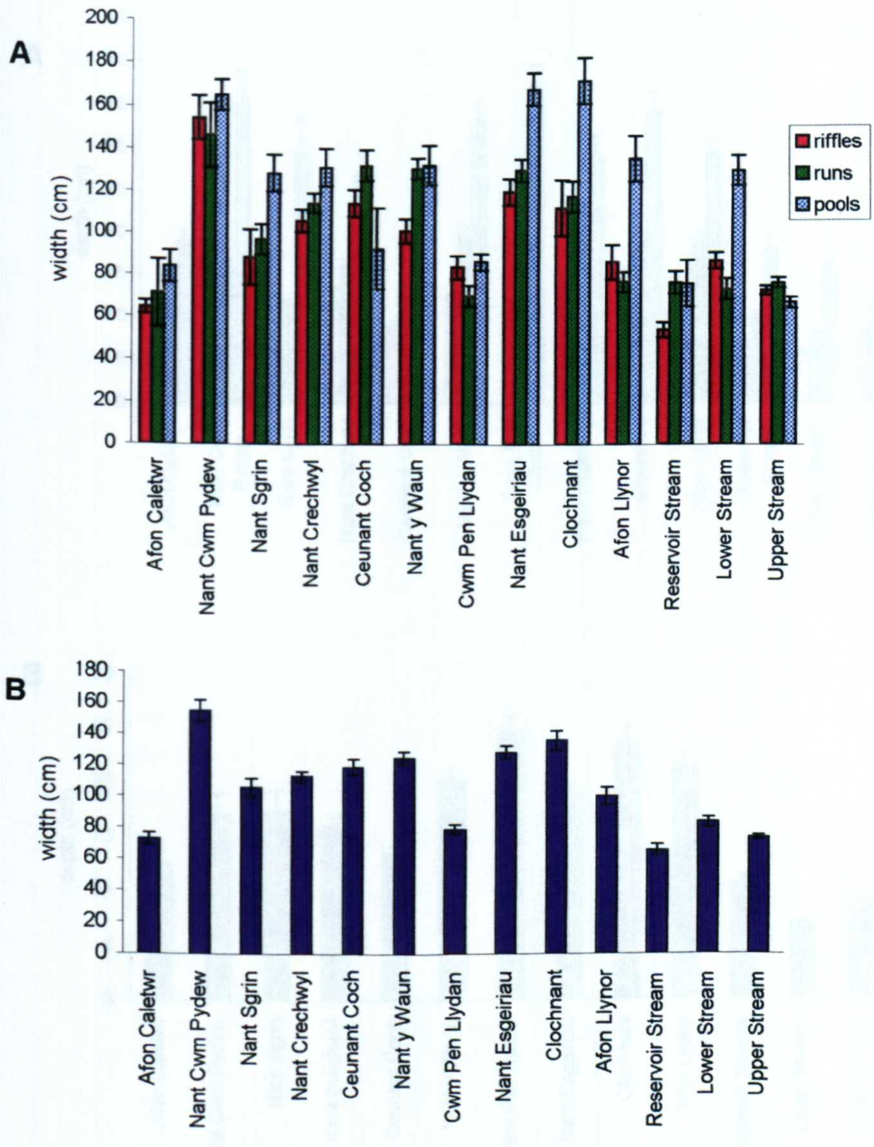


Fig.20 Mean width of stream habitats (A), and the entire streams (B) ±SEM.

n = (riffle, run, pool, stream): Cal (26, 3, 21, 50), NCP (28, 17, 15, 60), Sgr (10, 18, 15, 43), Cre (30, 44, 12, 86), Coch (21, 13, 4, 38); NYW (12, 41, 8, 61); CPL (23, 43, 47, 113); Esg (50, 40, 10, 100); Clo (10, 31, 24, 65); Lly (16, 40, 34, 90); Res (28, 20, 15, 63); Low (71, 26, 2, 99); Upp (62, 39,4, 105).

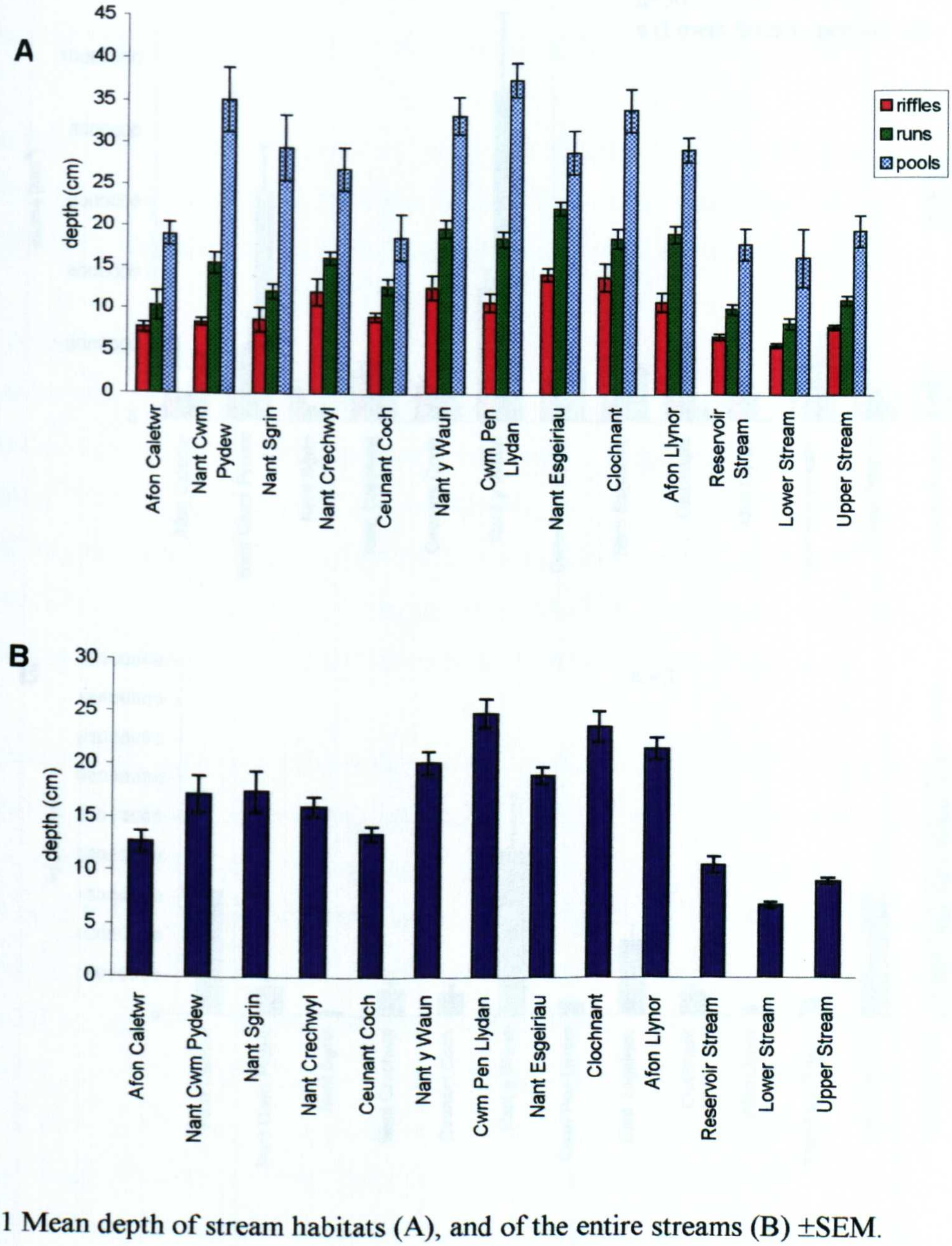


Fig.21 Mean depth of stream habitats (A), and of the entire streams (B) ±SEM.

n = (riffle, run, pool, stream): Cal (26, 3, 21, 50), NCP (28, 17, 15, 60), Sgr (10, 18, 15, 43), Cre (30, 43, 12, 85), Coch (21, 13, 4, 38); NYW (12, 41, 8, 61); CPL (23, 44, 47, 114); Esg (50, 40, 10, 100); Clo (10, 31, 24, 65); Lly (16, 40, 34, 90); Res (28, 20, 15, 63); Low (72, 26, 2, 100); Upp (68, 40,4, 112).

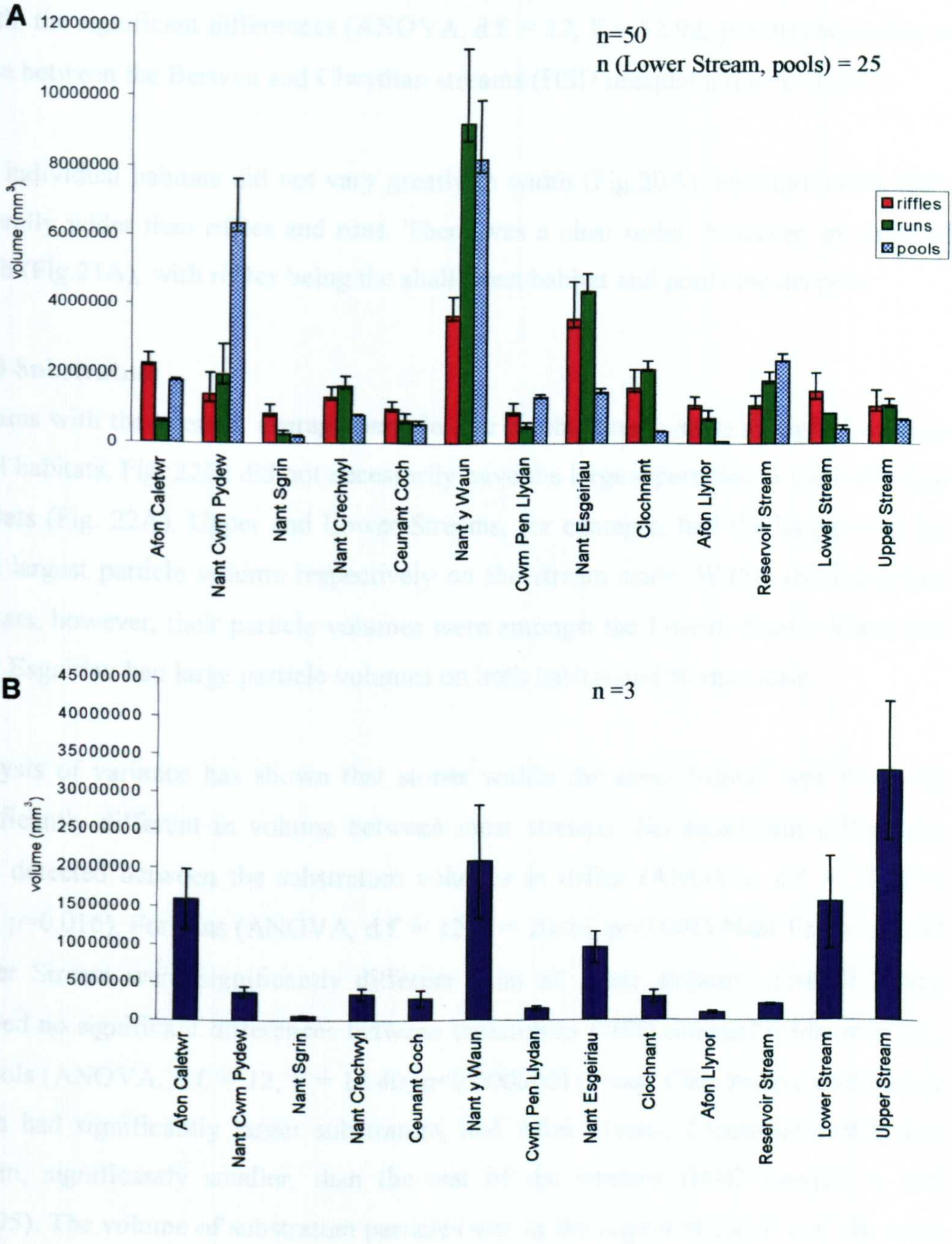


Fig. 22 Mean substratum particle volume in each of the three stream habitats (A), and in the stream as a whole (B) ± SEM.

11.4 cm±4.35SD (Ceunant Coch) to 24.9 cm±14.7SD (Cwm Pen Llydan). As for width, the significant differences (ANOVA, d.f. = 12, F = 32.92, $p<0.01$) were due to those between the Berwyn and Clwydian streams (HSD unequal n test $\alpha=0.05$).

The individual habitats did not vary greatly in width (Fig.20A), although pools were generally wider than riffles and runs. There was a clear order, however, in terms of depth (Fig.21A), with riffles being the shallowest habitat and pools the deepest.

2.6.3 Substratum

Streams with the greatest average particle size on the stream scale (weighed average of all habitats, Fig. 22B) did not necessarily have the largest particles in the individual habitats (Fig. 22A). Upper and Lower Streams, for example, had the largest and the third largest particle volume respectively on the stream scale. Within the individual habitats, however, their particle volumes were amongst the lowest. Nant y Waun and Nant Esgeiriau had large particle volumes on both habitat and stream scale.

Analysis of variance has shown that stones within the same habitat type were not significantly different in volume between most streams. No significant differences were detected between the substratum volumes in riffles (ANOVA, d.f. = 12, F = 5.80, $p=0.016$). For runs (ANOVA, d.f. = 12, F = 20.26, $p<0.001$) Nant Esgeiriau and Lower Stream were significantly different from all other streams, most of which showed no significant differences between themselves (HSD unequal n test $\alpha=0.05$). In pools (ANOVA, d.f. = 12, F = 14.40, $p<0.0000001$) Nant Cwn Pydew and Nant y Waun had significantly larger substratum, and Afon Llynor, Clochnant and Upper Stream, significantly smaller, than the rest of the streams (HSD unequal n test $\alpha=0.05$). The volume of substratum particles was in the region of $2x10^3$ cm^3 for most streams. The largest stones were found in the runs of Nant y Waun ($9.2x10^3$ cm^3), which is 138 times the volume of the smallest stones from the pools of Afon Llynor.

2.6.4 Shading and chlorophyll a

The proportion of PAR reaching the stream surface (Fig.23) varied from 0.14 to 0.42, except for the exposed streams such as Nant Cwm Pydew (0.79) and Nant y Waun (0.98) and the very shaded Ceunant Coch (0.07). The significant differences between the streams (ANOVA, d.f. = 12, F = 10.53, p<0.0000001) were largely due to those between these streams and the rest (HSD test α=0.05).

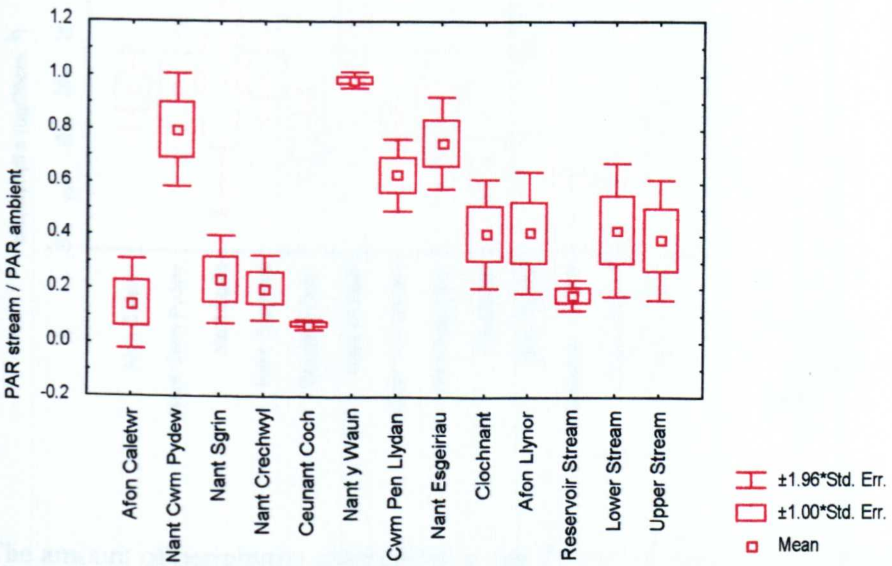


Fig.23 The mean proportion of photosynthetically active radiation penetrating to the stream surface (n=11).

The amount of chlorophyll on the stones (Fig.24) was not significantly different between the streams (ANOVA, d.f. = 12, F = 1.00, p=0.033). By far the lowest chlorophyll concentrations were in the two Clwydian streams: 4.3µg/25 cm^2±2.4SD in the Reservoir Stream, and 4.5µg/25 cm^2±2.9SD in the Lower Stream. The values for the Berwyn sites were, roughly five times greater, with the exception of Clochnant (7.4µg/25 cm^2±11.9SD). Variation around the means was, however very high.

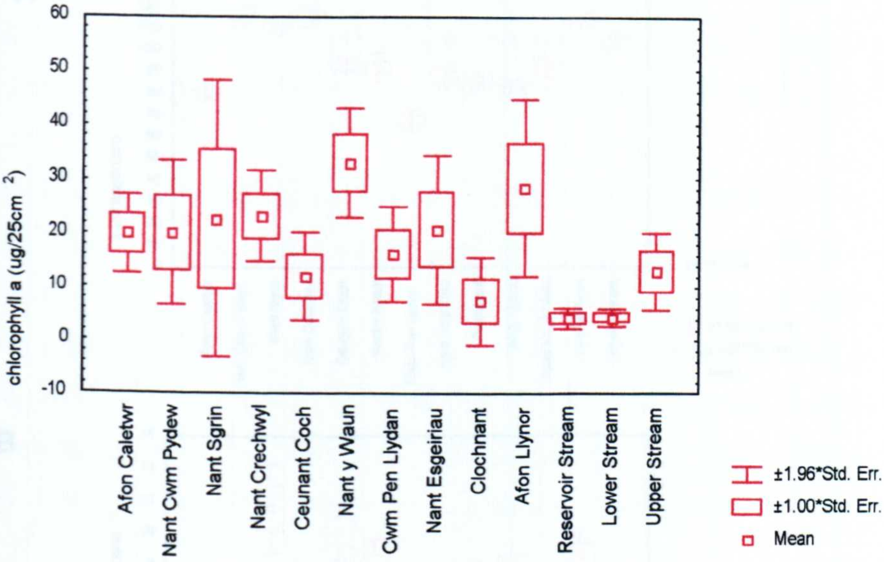


Fig.24 The amount of periphyton chlorophyll a per 25 cm^2 of the stream substratum. Annual mean (n = 9).

2.6.5 Bracken

The length of fronds in most catchments lay between 80 and 90 cm (Fig.25A), with the exceptions of Cwm Pen Lydan (67.1 cm±19.8SD). The differences in frond lengths were statistically significant (ANOVA, d.f. = 12, F = 35.70, $p<0.001$). Neighbouring streams Nant Cwm Pydew, Nant Sgrin and Nant Crechwyl formed a group with significantly higher fronds (unequal n HSD test, α=0.05), with height between 110 and 117cm. In the Clwyds the length of fronds in the catchment of Reservoir Stream (91.2 cm±22.5SD) was lower than that for Upper (104.1 cm±29.5SD) and Lower (113.2 cm±SD) streams.

In the Berwyns bracken frond density (Fig.25B) varied from 3.8±3.5SD fronds per 0.25m^2 in Afon Caletwr catchment to11.5±6.9SD fronds for Nant Sgrin and 10±4.3SD for Ceunant Coch. In the Clwyds Upper and Lower streams had densities of 6.3±3.1SD and 8.7±3.4SD fronds per 0.25m^2 respectively, whereas Reservoir stream had the second lowest density of all streams at 4.1±1.9SD fronds/0.25m^2. The differences in frond density were statistically significant (ANOVA, d.f. = 12, F =

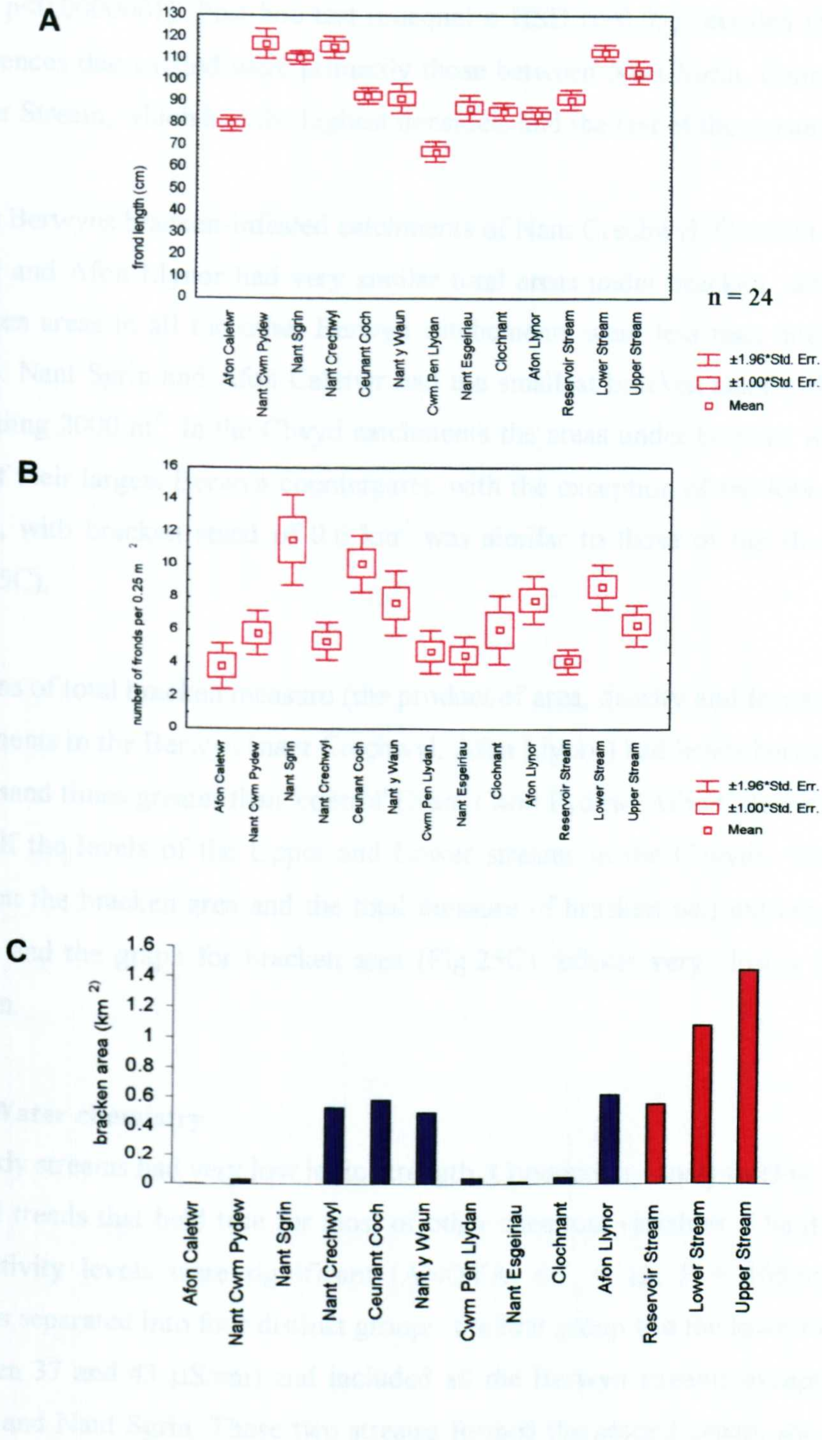


Fig.25 Bracken characteristics of study catchments. Mean frond length (A), frond density (B), and area under bracken (C).
(Frond length n = 92, 140, 280, 132, 249, 146, 71, 105, 148, 188, 98, 207, 121)

8.52, $p<0.0000001$). Post-hoc test (unequal n HSD $\alpha=0.05$) revealed that significant differences that existed were primarily those between Nant Sgrin, Ceunant Coch and Lower Stream, which had the highest densities, and the rest of the streams.

In the Berwyns bracken-infested catchments of Nant Crechwyl, Ceunant Coch, Nant y Waun and Afon Llynor had very similar total areas under bracken, around 0.5 km^2. Bracken areas in all the other Berwyn catchcments were less than one tenth of that figure. Nant Sgrin and Afon Caletwr had the smallest bracken stands, their areas not exceeding 2000 m^2. In the Clwyd catchments the areas under bracken were twice the size of their largest Berwyn counterparts, with the exception of the Reservoir stream, which, with bracken stand of 0.6 km^2 was similar to those of the Berwyn streams (Fig.25C).

In terms of total bracken measure (the product of area, density and frond height) some catchments in the Berwyn (nant Crechwyl, Afon Llynor) had levels between twenty to a thousand times greater than 'control' (Nant Cwm Pydew, Afon Caletwr) catchments, but half the levels of the Upper and Lower streams in the Clwyds. The correlation between the bracken area and the total measure of bracken was extremely high ($r = 0.956$) and the graph for bracken area (Fig.25C) reflects very closely that for total bracken.

2.6.6 Water chemistry

All study streams had very low ionic strength. Conductivity analysis (Fig.26) revealed general trends that held true for most of other chemical variables. The differences in conductivity levels were significant (ANOVA, d.f. = 12, $F = 105.95$, $p<0.001$). Streams separated into four distinct groups: the first group had the lowest conductivity (between 37 and 43 $\mu S/cm$) and included all the Berwyn streams except Nant Cwm Pydew and Nant Sgrin. These two streams formed the second group, showing higher conductivity: 53.7 and 55.7$\mu S/cm$ respectively. Clwydian streams were far more ion-rich. Conductivity of Lower and Upper streams was more than 4 times greater than that of most of the Berwyn streams. Reservoir Stream conductivity lay approximately half way between the Clwyd and Berwyn streams, and was significantly different from all other streams (unequal n HSD test, $\alpha=0.05$).

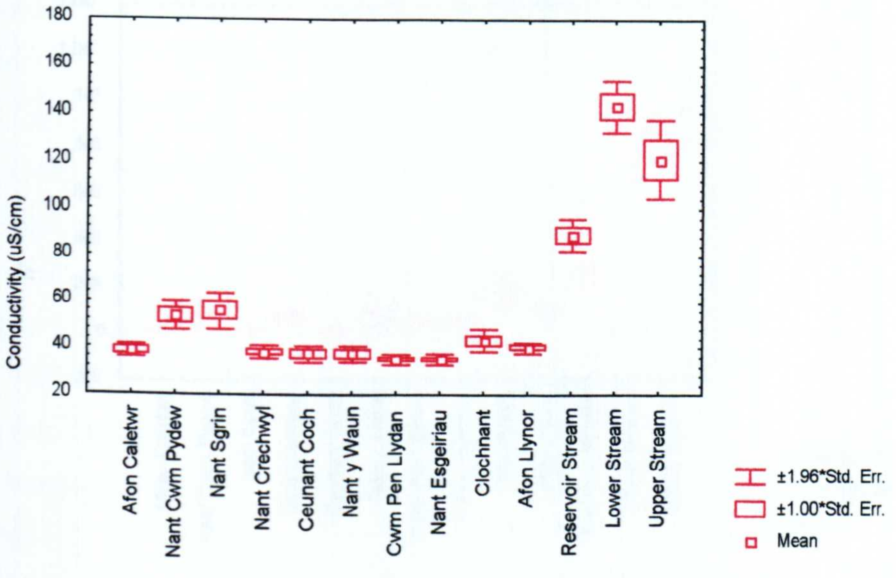


Fig.26 Conductivity of study streams. The three rightmost are the Clwydian streams.(n=12).

2.6.6.1 Acidity

All study streams were acidic, with mean annual pH below 7 (Fig.27, Table 3). In the Berwyn pH was below 6 for all streams except Clochnant. The most acidic stream was Ceunant Coch with pH of 5.11. The Clwydian streams were less acidic. Mean annual pH of the Lower and the Upper streams was approaching neutrality. Significant differences reported by analysis of variance (ANOVA, d.f. = 12, F = 30.63, p<0.001) were limited to those between these two streams and the rest, which were not significantly different between themselves (HSD test, α=0.05). Reservoir stream, the pH of which (6.4) was closer to Berwyn streams than to the other two Clwydian ones.

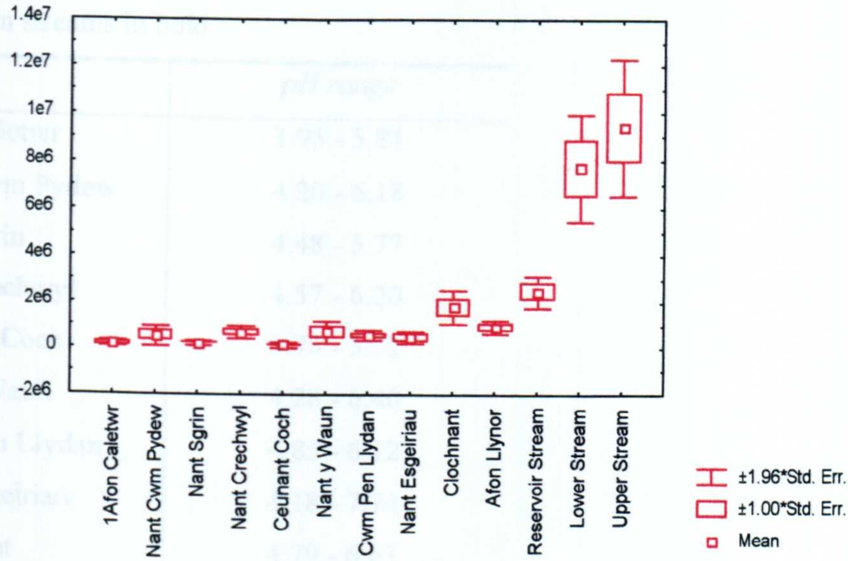


Fig. 27 Mean annual acidity of the study streams expressed as concentrations of H^+. (n=12). (Note: in the y-axis units 2e6 denotes 2^6).

An important chemical variable in upland streams is the lowest pH experienced (Table.3). Within the Berwyn sites, Ceunant Coch, the most acidic stream, experienced the lowest minimum pH of 3.78, closely followed by Afon Caletwr, Nant Cwm Pydew and Nant Sgrin. Nant Crechwyl, sandwiched between Nant Sgrin and Ceunant Coch had much higher minimum pH of 4.57 than the neighbouring streams. The less acidic Clochnant and Afon Llynor also had the highest minimum pH: their lowest recorded values were 4.79 and 5.13 respectively. In the Clwyds Reservoir stream had minimum pH of 5.87, compared to 6.41 and 6.5 of the Lower and Upper streams.

Another difference between Berwyn and Clwyd streams was greater amplitude of change in pH levels in the former group. During the 12 month sampling period the pH values in the Clwyds fluctuated within just one pH unit, between 6 and 7. In the Berwyns, however, the range of pH values for several streams extended across two pH units.

Table 3. pH profile of study streams based on monthly samples.
Clwydian streams in bold

Stream	*pH range*
Afon Caletwr	3.95 - 5.81
Nant Cwm Pydew	4.20 - 6.18
Nant Sgrin	4.48 - 5.77
Nant Crechwyl	4.57 - 6.20
Ceunant Coch	3.78 - 5.72
Nant y Waun	4.28 - 6.40
Cwm Pen Llydan	4.85 - 6.12
Nant Esgeiriau	4.18 - 7.74
Clochnant	4.79 - 6.63
Afon Llynor	5.13 - 6.22
Reservoir Stream	5.87 - 6.71
Lower Stream	6.41 - 7.20
Upper Stream	6.50 - 7.24

2.6.6.2 Sodium and chloride

The differences between sodium concentrations (Fig.28A) were statistically significant (ANOVA, d.f. = 12, F = 22.08, $p<0.0000001$). The concentrations of sodium in the Berwyn streams were very similar (differences not significant, HSD test, $\alpha=0.05$) and lay between 4.1 and 4.8mg/l, except for Nant Cwm Pydew and Nant Sgrin. The annual means in these two streams were higher: 7.57 and 8.05 mg/l respectively. These two groups were significantly different from each other (HSD test, $\alpha=0.05$). In the Clwyds the Reservoir Stream had lower (8.15mg/l) concentration than the Lower (9.8mg/l) and Upper (9.76 mg/l) Streams. This difference was statistically significant (HSD test, $\alpha=0.05$). The levels of chloride had an identical pattern to sodium (Fig.28B), with significant differences (ANOVA, d.f. = 12, F = 9.76, $p<0.0000001$; HSD test, $\alpha=0.05$) between the same streams. In the Berwyn the chloride levels lay between 10.6 and 15.6mg/l, with Clwyds showing higher levels: between 17 and 18.9 mg/l.

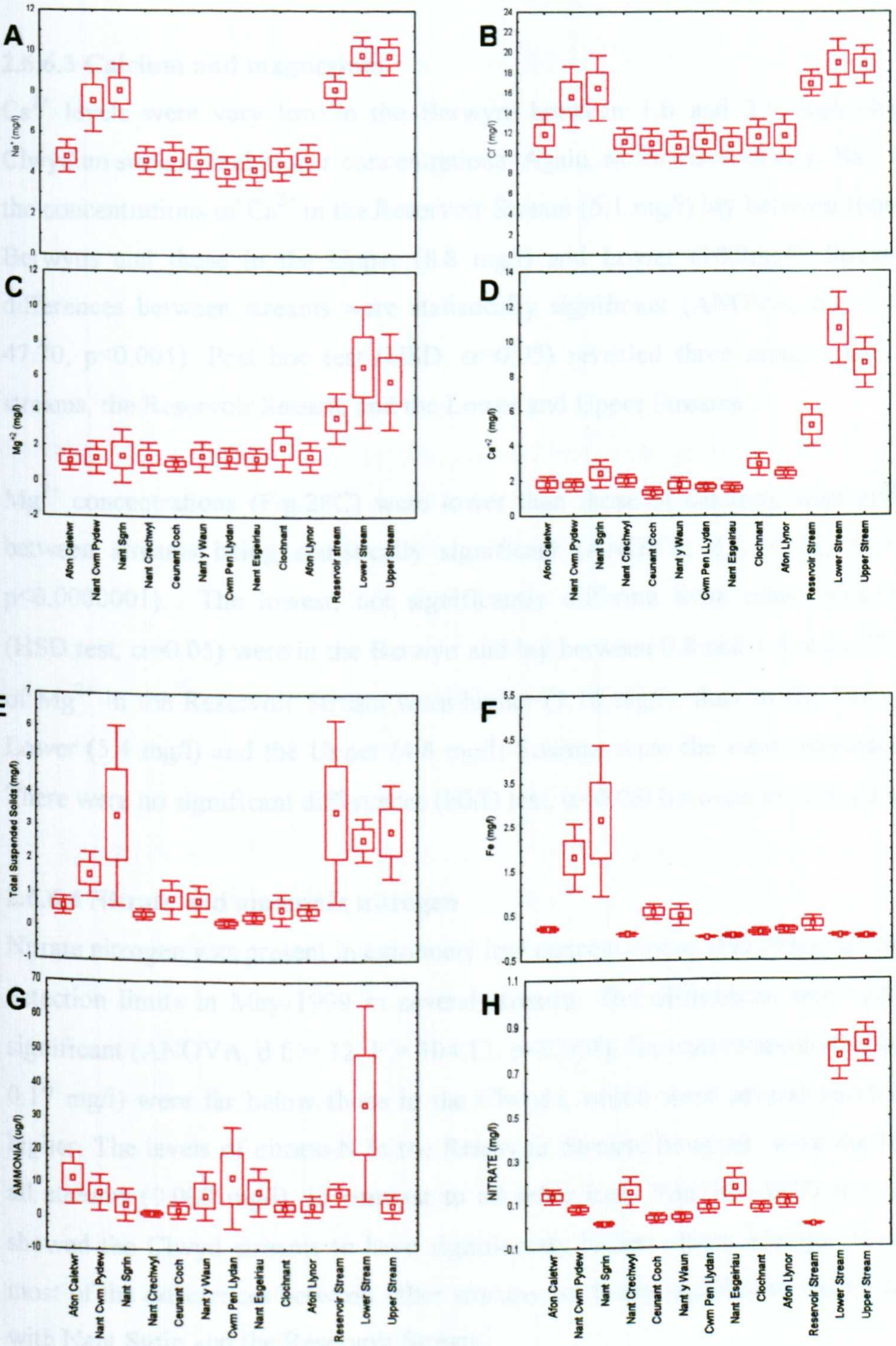


Fig.28 Annual mean concentration of chemical variables in the study streams. (Note: Ammonia and nitrate represent NH_4-N and NO_3-N)

±1.96*Std. Err. ±1.00*Std. Err. Mean (n=12)

2.6.6.3 Calcium and magnesium

Ca^{2+} levels were very low in the Berwyn, between 1.8 and 2.9 mg/l (Fig.28D). Clwydian streams had higher concentrations. Again, as for conductivity, Na^{+}, and Cl^{-}, the concentrations of Ca^{2+} in the Reservoir Stream (5.1 mg/l) lay between those in the Berwyns and those in the Upper (8.8 mg/l) and Lower (10.8mg/l) Streams. The differences between streams were statistically significant (ANOVA, d.f. = 12, F = 47.70, $p<0.001$). Post hoc test (HSD, $\alpha=0.05$) revealed three groups: the Berwyn streams, the Reservoir Stream, and the Lower and Upper Streams.

Mg^{2+} concentrations (Fig.28C) were lower than those of calcium, with differences between streams being statistically significant (ANOVA, d.f. = 12, F = 14.69, $p<0.0000001$). The lowest, not significantly different from other concentrations (HSD test, $\alpha=0.05$) were in the Berwyn and lay between 0.8 and 1.4 mg/l. The levels of Mg^{2+} in the Reservoir Stream were higher (3.16 mg/l), than in the Berwyn. The Lower (5.4 mg/l) and the Upper (4.8 mg/l) Streams were the most magnesium-rich. There were no significant differences (HSD test, $\alpha=0.05$) between the Clwyd streams.

2.6.6.4 Nitrate and ammonia nitrogen

Nitrate nitrogen was present in extremely low concentrations (Fig.28H), falling below detection limits in May 1999 in several streams. The differences were statistically significant (ANOVA, d.f. = 12, F = 104.13, $p<0.001$). Berwyn concentrations (0.07 to 0.17 mg/l) were far below those in the Clwyds, which were several hundred times higher. The levels of nitrate-N in the Reservoir Stream, however, were the lowest of all streams (0.008 mg/l), in contrast to all other ions. Post hoc HSD test ($\alpha=0.05$) showed the Clwyd streams to have significantly higher nitrate nitrogen levels, with most of the differences between other streams not being significant, except for those with Nant Sgrin and the Reservoir Stream.

Ammonia N (Fig.28G) was another scarce determinand, not detectable in the study streams in May 1999 and June 2000. It was below detectable limits on several other occasions in all streams, but not at the same time. Ammonia nitrogen showed episodic behaviour with sudden surges in concentration. For example in Cwm Pen Llydan, it peaked at 93.21µg/l in October 1999, a 12 fold increase on the September levels, but had been undetectable in August. The Lower Stream, which had the highest ammonia nitrogen level of 34.2 μg/l experienced an even higher peak of 102 μg/l in October 1999. The statistically significant differences (ANOVA, d.f. = 12, F = 104.13, p=0.0022) in ammonia nitrogen levels among streams were due to that between the Lower stream, and the rest, which were not significantly different between themselves (HSD test, α=0.05).

2.6.6.5 Iron, total suspended solids, ptaquiloside

Unlike most other chemical determinands, the concentrations of iron in the Clwydian streams were similar to those in most of the Berwyn streams (Fig.F). The two streams with much higher concentrations were Nant Cwm Pydew (1.83 mg/l) and Nant Sgrin (2.67 mg/l). The significant differences (ANOVA, d.f. = 12, F = 8.95, p<0.0000001) between the streams were due to these two streams being significantly different from the rest (apart from Ceunant Coch) which were not significantly different amongst themselves (HSD test, α=0.05).

The amount of total suspended solids (TSS) in the streams followed the same pattern as most chemical species in the study streams (Fig.28E). Clwyd streams showed higher levels than the Berwyn streams out of which Nant Sgrin (3.32 mg/l) and Nant Cwm Pydew (1.54 mg/l) had concentrations higher than the rest. Significant differences (ANOVA, d.f. = 12, F = 4.13, p=0.000015) between streams were generally restricted (HSD test, α=0.05) to those between the Reservoir Stream, which had the greatest amount of TSS (3.47 mg/l) and the streams with the lowest concentrations, such as Cwm Pen Llydan (0.13 mg/l).

Neither ptaquiloside, nor its breakdown products, pterosins were detected in any of the samples from the study streams.

2.7 Discussion

2.7.1 Significance of physical variables

The length of study streams, the size of their catchment areas and altitude of sampling points were an artefact of the selection process of study streams. The Clwydian Range is a single chain of mountains with a narrow moorland ridge, where a short valley under upland vegetation quickly gives way to improved grassland, woodland and farmland. The Berwyns are a much wider mountain range, with wider, longer valleys, where moorland vegetation is not restricted to a narrow band on the watershed but spills down into the valleys. The length of the study streams was determined by the location of the cut-off point. In the Clwyds this point was at the end of upland vegetation. As Clwydians are lower than the Berwyns, the study streams there were situated at lower altitude. The length of the Berwyn streams was also largely determined by the vegetation change, but within moorland. For several streams the cut-off was the start of coniferous plantations (Nant Cwm Pydew, Nant Crechwyl, Ceunant Coch, Nant Sgrin), for some (Afon Caletwr) it was the appearance of bracken. Nant Esgeiriau, Clochnant, flowing down long moorland valleys with no conifer plantations had greater length and catchment area than other streams.

Stream width is a measure of the total area of stream bed available for stream biota, and can be a limiting factor, especially for territorial organisms. In this study the streams were divided into habitats and invertebrates sampled from particular habitat types: riffles and pools. When measuring physiochemical variables, which influence the invertebrate community structure, two approaches can be used: measuring all variables on the stream scale or on the habitat scale. In case of stream width, this variable for all three habitat types was significantly correlated with the stream mean, and therefore the stream mean can be used for further analysis. The same was observed for stream depth.

Substratum is one of the key factors in running water ecosystems (See Introduction, Chapter 3). All streams contained boulders (diameter > 256mm, (Cummins, 1962)), and in several streams boulders were the dominant substratum size category. The flow was seen to be turbulent, but unfortunately the measure of current velocity and hence discharge was not made. This was due to equipment failure, when this problem was rectified the petrol shortages and then the foot and mouth epidemic prevented access to the streams at the time when maximum (February) and minimum (August - September) discharge could be measured.

Contradicting visual observations the average particle volume in pools was not less, and in some cases was greater than that in riffles and runs. This is due to great variation in substratum composition in pools. Despite the majority of the substratum particles being much smaller in pools than in riffles or runs, picking out just one of larger particles drastically increases the pool mean. In some pools, such as those in Nant y Waun and Reservoir Stream, there are very large boulders on the periphery of the pools with a gravel centre in the middle. Even though there is a much greater number of gravel and sand particles in such pools, the area they occupy is less then that of boulders, and they are, therefore, underrepresented in the sample. It is reasonable, therefore, to conclude that pools in turbulent upland streams do not fit the classic description as the small substratum particles are rapidly washed down the stream, with few beds of pure gravel or sand.

2.7.2 Water chemistry

The results of water chemistry analysis agree with previous work on upland stream chemistry ((Reynolds *et al.*, 1989; Robson & Neal, 1996). Clwydian streams, situated at lower altitude than the Berwyn ones (and also due their different geology) show more lowland characteristics - greater ionic strength and buffering capacity and lower acidity. Agricultural activity in the Clwyds is reflected in higher amount of nitrate nitrogen than in the Berwyns. The influence of the sea was stronger in the Clwydian streams, where sodium and chloride, ions of marine origin, were found at concentrations which were double then those in the Berwyns.

Intimate relationship between small upland streams and their catchments is illustrated by significant differences in several water chemistry variables between neighbouring catchments. Two adjacent streams: Nant Sgrin and Nant Cwm Pydew, have higher ionic strength than neighbouring streams less than a kilometre away. This is attributable to small-scale differences in soil and geology, too fine to be seen on soil maps. Similarly, Reservoir Stream, situated a kilometre away from the other two Clwydian streams, is markedly different from them in terms of water chemistry. It shows stronger 'upland' characteristics, such as lower ionic strength and pH, lower concentrations of Mg^{+2} and Ca^{+2} and very low concentrations of nitrate nitrogen. Again, the reasons are small-scale geological and topographical differences, and lesser human involvement in the Reservoir Stream catchment.

Lack of ptaquiloside in the stream water can be attributed to the fact that this metabolite is not leached from fronds, but it is also possible that it is too unstable and does not survive the passage through soils before reaching the stream. Instability of ptaquiloside in acidic conditions lends credibility to the latter. Pterosins, the breakdown products of ptaquiloside, were not detected either. It is likely, therefore, that ptaquiloside is not removed from live, undamaged fronds by rain. When the outer cuticle of fronds is brocken, ptaquiloside is removed by water. During thin layer chromatography pterosin spots were obtained using water in which crushed fronds had been soaked. No corresponding spots were seen when stream water was used. However, only one sample from each stream was tested and pterosin levels may not necessarily be detectable all year round, similarly to nitrate and ammonium nitrogen.

2.7.3 Bracken

In the Berwyn bracken follows the distribution of suitable soils. The catchments whose primary soil-type is dry well-aerated brown podzolic soils (Nant Crechwyl), have large continuous stands of bracken. Where dominant soils are peaty and wet, such as ferric stagnopodzols or stagnohumic gleys, bracken is confined to islands of suitable soils which vary in size (Nant Sgrin, Nant Cwm Pydew).

Differences in frond height between catchments can be attributed to several factors. Altitude is significantly negatively correlated with frond length ($r=-0.551$; $p<0.05$), which agrees with the work of Atkinson (1989), who stated that at higher altitudes canopy height is reduced. However, he also states that this is accompanied by an increase in frond density. No corresponding increase in frond density was observed in the study catchments, and neither was frond density correlated with frond height ($r = 0.389$). The streams that have the tallest fronds appear on the steepest valleys - Nant Cwm Pydew, Nant Crchwyl, Upper Stream, Lower Stream. These stands are old and have not been subjected to control measures, due to inaccessibility of the valleys (Upper and Lower Streams), but also to prevent landslides (Nant Cwm Pydew). Bracken has been subjected to control measures in the past in the catchments of Esgeiriau and Clochnant. Another reason may be a temporal one. Whereas in some catchments bracken invasion successfully occurred several decades ago, it may be in earlier stages in other catchments. This appears appropriate to Nant Esgeiriau and Clochnant, which have suitable soils, but bracken stands are limited in area, and plants of other species are mixed with bracken in the stands. However, this invasion may also be kept in check by the higher altitude of the Esgeiriau and Clochnant catchments. In the Clwydians, Reservoir Stream also seems to be at an earlier successional stage towards bracken dominance than Upper and Lower Streams.

2.7.4 Habitat classification

Differences between the habitat structure of study streams set the Clwydian streams apart from the Berwyn ones. The former lack cascades and sheets, have virtually no pools and are dominated by riffles. In most Berwyn streams, all habitat types are represented relatively evenly. Berwyn streams, therefore, have higher habitat complexity with high local variation in flow and substratum. Variation between streams also exists in the Berwyn, with Nant Pen Llydan a slow-flowing stream with a flat stream bed, dominated by pools and runs with few cascades. In contrast, Nant Cwm Pydew has extensive sheets and cascades and many riffles. Habitat structure has also highlighted the difference between the Reservoir Stream and the two Clwydian ones, as the former has a much more complex habitat structure, and is more similar to the Berwyn streams than to its Clwydian counterparts.

2.7.5 Correlated variables

Many of the physiochemical variables were significantly correlated with one or several others. See Tables 4, 5 & 6. Chemical variables especially showed a high degree of intercorrelation, which is the norm in upland streams (Sutcliffe & Hildrew, 1989). In the Berwyns, conductivity (Table.4) was correlated with Cl^-, Fe, and Na^+, all of which were also significantly positively intercorrelated. It was not correlated with Ca^{+2} and Mg^{+2}. When the data from Berwyn and Clwyd were pooled, the greater ionic strength of Clwyds extended the correlation between conductivity and other ions to Ca^{+2}, Mg^{+2}, NO_3-N and NH_4-N and also resulted in intercorrelation between most of these ions, which did not exist in the Berwyn.

Inclusion of low-altitude Clwydian streams into the analysis extends the upland - lowland gradient within the study streams and introduces several correlations (Table.4) which are not themselves significant in the Berwyns (Table.6). Altitude, for example, is thus significantly negatively correlated with almost all the chemical variables when Berwyn and Clwyd data are pooled. Such a relationship is valid, as it fits with the general continuum of the upland - lowland change in chemical characteristics. Clwydian streams, however, are also shallower and more narrow than the Berwyn ones, with fewer pools and cascades, despite being situated at lower altitude. This creates a coincidental, but statistically significant correlation between percentage of riffles and pools and chemical variables.

In the Berwyns, longer streams with larger catchments (Nant Esgeiriau, Clochnant), were deeper with fewer cascades, more pools and had greater concentrations of magnesium. Shorter, shallower, faster-flowing streams with smaller catchments had greater concentrations of iron, more cascades and riffles and higher acidity. All these correlations are statistically significant (Table.6). The chemical differences are probably due to the greater amount of buffering ions available in larger catchments. Also, larger streams are not as dependent on rain water, which is the main source of acidity, relying more on less acidic groundwater.

Bracken area in the Berwyn is negatively correlated with altitude. However, in pooled data (Berwyn and Clwyd), altitude is also highly correlated with pH, and all other chemical variables, except for Cl^- and Fe. Therefore bracken area (and hence total bracken as the two are also highly correlated) is positively correlated with all chemical variables associated with acidity. This relationship, however, did not exist in the Berwyn. See Chapter 3 for the discussion of the correlations between bracken frond density and height and the chemical variables.

Table 4. Pearson product-moment coefficients of correlations between water chemistry variables.(* $p<0.05$; ** $p<0.01$; *** $p<0.001$)

Clwyd and Berwyn

	pH	Ca^{+2}	Mg^{+2}	Alk	Cond	NO_3-N	NH_4-N	Cl^-	Fe	Na^+
Ca^{+2}	0.863***									
Mg^{+2}	0.881***	0.995***								
Alk	0.909***	0.969***	0.965***							
Cond	0.813***	0.982***	0.981***	0.927***						
NO_3-N	0.753**	0.888***	0.880***	0.920***	0.837***					
NH_4-N	0.391	0.616*	0.595*	0.533*	0.598*	0.535*				
Cl^-	0.627*	0.821***	0.817***	0.755**	0.902***	0.636*	0.413			
Fe	-0.436	-0.253	-0.280	-0.285	-0.114	-0.347	-0.203	0.269		
Na^+	0.607*	0.819***	0.815***	0.753**	0.903***	0.650*	0.404	0.993***	0.290	
TSS	0.436	0.643*	0.647*	0.543	0.750**	0.364	0.210	0.911***	0.422	0.911***

Berwyn

	pH	Ca^{+2}	Mg^{+2}	Alk	Cond	NO_3-N	NH_4-N	Cl^-	Fe	Na^+
Ca^{+2}	0.579*									
Mg^{+2}	0.816***	0.854***								
Alk	0.872***	0.831***	0.920***							
Cond	-0.106	0.433	0.232	0.172						
NO_3-N	0.239	-0.073	-0.047	-0.006	-0.481					
NH_4-N	-0.166	-0.409	-0.215	-0.294	-0.225	0.213				
Cl^-	-0.256	0.289	0.070	0.031	0.968***	-0.431	-0.075			
Fe	-0.393	0.136	-0.046	-0.095	0.913***	-0.629*	-0.162	0.937***		
Na^+	-0.325	0.190	-0.026	-0.053	0.957***	-0.492	-0.139	0.981***	0.973***	
TSS	-0.412	0.246	0.020	-0.038	0.868***	-0.634*	-0.227	0.886***	0.967***	0.914***

Table 5. Pearson product - moment coefficients of correlations between physiochemical variables measured as part of the survey in both Clwydian and Berwyn streams. (Significant coefficients highlighted. Black: $p<0.05$; blue: $p<0.01$; red: $p<0.001$)

	Alt	Length	Ct Area	Depth	Width	Moss	Casc	Riffle %	Run %	Pool %	Shade	Chlrph	RiffleV	RunV	PoolV	StreamV	Pt length	Pt dens	Pt area	Pt total
Length	0.366																			
Ct Area	0.307	**0.769**																		
Depth	**0.807**	0.396	**0.573**																	
Width	0.481	0.140	0.480	0.475																
Moss	-0.344	0.479	0.164	-0.332	-0.283															
Casc	0.250	-0.188	0.105	0.215	0.454	-0.277														
Riffle %	**-0.640**	-0.164	-0.456	**-0.858**	-0.299	0.326	-0.481													
Run %	0.072	0.061	0.521	0.377	0.404	-0.248	0.328	-0.490												
Pool %	**0.637**	0.127	0.055	0.616	-0.017	-0.144	0.246	**-0.675**	-0.313											
Shade	0.296	0.268	0.466	0.451	0.475	-0.225	-0.105	-0.231	0.418	-0.103										
Chlrph	0.520	-0.073	0.120	0.479	0.334	-0.360	0.054	-0.507	0.418	0.198	0.428									
RiffleV	0.327	0.446	0.473	0.165	0.327	0.066	-0.165	-0.070	0.358	-0.228	**0.603**	0.431								
RunV	0.199	0.224	0.500	0.253	0.405	-0.112	0.141	-0.292	**0.728**	-0.298	**0.715**	0.471	**0.840**							
PoolV	0.235	-0.202	-0.013	0.140	0.401	-0.343	0.298	-0.172	0.404	-0.155	**0.699**	0.430	**0.540**	**0.756**						
StreamV	-0.487	0.082	-0.066	-0.477	-0.285	**0.589**	-0.503	0.489	0.014	**-0.544**	0.219	0.028	0.377	0.334	0.218					
Pt length	**-0.551**	-0.524	-0.243	-0.520	0.271	0.055	0.196	0.400	0.074	-0.498	-0.061	-0.033	-0.162	-0.034	0.115	0.151				
Pt dens	-0.188	-0.192	0.122	-0.090	0.191	-0.109	0.415	-0.044	0.182	-0.106	-0.168	0.098	-0.273	-0.104	-0.171	-0.101	0.389			
Pt area	**-0.879**	-0.317	-0.224	**-0.645**	-0.423	0.228	-0.353	**0.532**	0.096	**-0.661**	-0.164	-0.262	-0.221	-0.058	-0.170	**0.610**	0.362	0.209		
Pt total	**-0.854**	-0.315	-0.197	**-0.660**	-0.339	0.177	-0.384	**0.616**	0.026	**-0.693**	-0.143	-0.296	-0.187	-0.085	-0.198	**0.581**	0.396	0.342	**0.956**	
pH	**-0.767**	-0.029	0.072	-0.389	-0.341	0.364	-0.384	0.342	0.001	-0.374	0.116	-0.479	-0.132	0.007	-0.116	0.485	0.236	-0.149	**0.722**	**0.654**
Ca^{+2}	**-0.913**	-0.154	-0.171	**-0.713**	-0.494	0.436	-0.414	**0.651**	-0.268	-0.481	-0.133	**-0.587**	-0.234	-0.213	-0.260	**0.543**	0.387	0.110	**0.785**	**0.804**
Mg^{+2}	**-0.916**	-0.129	-0.161	**-0.710**	-0.505	0.444	-0.416	**0.652**	-0.232	-0.514	-0.100	**-0.600**	-0.211	-0.173	-0.220	**0.564**	0.360	0.061	**0.791**	**0.796**
Alk	**-0.871**	-0.107	-0.067	**-0.569**	-0.422	0.466	-0.463	**0.538**	-0.161	-0.450	-0.019	-0.475	-0.216	-0.154	-0.243	**0.596**	0.373	0.106	**0.799**	**0.805**
Cond	**-0.919**	-0.221	-0.255	**-0.768**	-0.476	0.398	-0.315	**0.686**	-0.298	-0.495	-0.138	**-0.599**	-0.276	-0.232	-0.187	0.503	0.464	0.135	**0.750**	**0.762**
NO_3-N	**-0.787**	-0.049	-0.145	**-0.623**	-0.382	0.515	-0.671	**0.723**	-0.268	**-0.561**	-0.018	-0.368	-0.100	-0.185	-0.272	0.719	0.358	0.051	**0.781**	**0.824**
NH_4-N	-0.395	-0.124	-0.168	-0.420	-0.325	-0.026	-0.482	**0.533**	-0.419	-0.226	0.128	-0.388	0.078	-0.096	-0.038	0.223	0.088	0.013	0.229	0.411
Cl^-	**-0.785**	-0.288	-0.351	**-0.700**	-0.387	0.367	-0.062	**0.566**	-0.385	-0.290	-0.169	-0.508	-0.419	-0.342	-0.124	0.327	**0.573**	0.205	**0.538**	0.516
Fe	0.193	-0.219	-0.105	0.063	0.362	-0.130	**0.655**	-0.185	-0.053	0.247	0.021	0.240	-0.211	-0.096	0.224	-0.315	0.459	**0.541**	-0.379	-0.342
Na^+	**-0.808**	-0.328	-0.362	**-0.735**	-0.347	0.351	-0.042	**0.594**	-0.329	-0.369	-0.149	-0.464	-0.380	-0.286	-0.070	0.374	**0.632**	0.256	**0.577**	**0.563**
TSS	**-0.693**	-0.249	-0.280	**-0.651**	-0.390	0.338	0.226	0.373	-0.207	-0.232	-0.271	-0.436	-0.376	-0.230	-0.076	0.200	0.520	0.317	0.418	0.359

Alt: altitude; length: stream length; Ct area: catchemnt area; casc: cascade and sheet length; Riffle, pool, run, stream V: volume of substratum particles; Pt: bracken.

NOTE: an increase in variable 'shade' actually represents a decrease in shading.

Table 6. Pearson product - moment coefficients of correlations between physiochemical variables measured as part of the survey in the Berwyn streams. (Significant coefficients highlighted. Black: p<0.05; blue: p<0.01; red: p<0.001)

	Alt	*Length*	*Ct Area*	*Depth*	*Width*	*Moss*	*Casc*	*Riffle %*	*Run %*	*Pool %*	*Shade*	*Chlrph*	*RiffleV*	*RunV*	*PoolV*	*StreamV*	*Pt length*	*Pt dens*	*Pt area*	*Pt total*
Length	**0.616**																			
Ct Area	0.226	**0.782**																		
Depth	0.392	0.415	**0.620**																	
Width	-0.150	0.059	0.404	0.056																
Moss	0.229	**0.610**	0.352	-0.090	-0.081															
Casc	-0.435	-0.275	0.090	-0.119	0.539	-0.019														
Riffle %	0.031	-0.078	-0.480	**-0.770**	0.063	0.051	-0.209													
Run %	-0.445	-0.003	0.518	0.307	0.399	-0.279	0.265	-0.449												
Pool %	0.411	0.074	-0.074	0.399	-0.452	0.230	-0.071	-0.469	**-0.579**											
Shade	0.452	0.248	0.429	0.528	0.437	-0.266	-0.081	-0.267	0.438	-0.190										
Chlrph	-0.155	-0.332	-0.092	-0.044	-0.104	-0.431	-0.114	-0.253	0.377	-0.143	0.401									
RiffleV	0.323	0.451	0.432	-0.024	0.215	0.261	-0.275	0.092	0.353	-0.433	**0.585**	0.401								
RunV	0.068	0.205	0.489	0.178	0.403	-0.043	0.063	-0.228	0.723	-0.506	**0.738**	0.512	**0.849**							
PoolV	0.130	-0.243	-0.048	-0.011	0.423	-0.326	0.206	-0.007	0.371	-0.361	**0.750**	0.482	0.544	**0.748**						
StreamV	0.232	0.113	0.046	-0.232	-0.058	0.198	-0.193	0.106	0.208	-0.303	0.383	0.448	**0.856**	**0.749**	**0.638**					
Pt length	**-0.677**	-0.530	-0.211	-0.450	**0.578**	-0.136	**0.567**	0.180	0.211	-0.373	-0.063	0.216	-0.124	0.044	0.238	-0.162				
Pt dens	-0.492	-0.201	0.084	-0.146	0.140	-0.140	**0.779**	-0.234	0.275	-0.059	-0.285	0.084	-0.366	-0.094	-0.133	-0.314	0.358			
Pt area	**-0.627**	-0.471	-0.088	-0.130	0.021	**-0.620**	0.122	-0.141	0.552	-0.417	-0.152	0.393	-0.067	0.191	0.027	0.015	0.130	0.350		
Pt total	-0.611	-0.431	-0.106	-0.228	0.052	**-0.582**	0.207	-0.015	0.508	-0.489	-0.215	0.264	-0.088	0.154	0.013	0.008	0.115	0.434	**0.974**	
pH	0.113	0.190	0.551	**0.778**	0.332	-0.073	-0.091	**-0.669**	0.393	0.222	0.475	0.050	0.131	0.318	0.109	-0.079	-0.118	-0.336	0.078	-0.074
Ca^{+2}	-0.078	0.115	0.364	0.416	0.111	0.379	0.306	**-0.662**	-0.046	**0.650**	-0.159	-0.126	-0.256	-0.177	-0.331	-0.317	0.099	0.152	-0.147	-0.251
Mg^{+2}	0.132	0.363	**0.652**	**0.728**	0.278	0.364	0.203	**-0.761**	0.225	0,472	0.255	-0.167	0.010	0.157	-0.079	-0.162	-0.066	-0.061	-0.254	-0.360
Alk	-0.067	0.084	0.480	**0.744**	0.150	0.074	0.125	**-0.867**	0.323	0.472	0.224	0.066	-0.122	0.113	-0.082	-0.239	0.000	-0.039	0.004	-0.157
Cond	-0.130	-0.271	-0.139	-0.106	0.381	0.077	**0.683**	-0.091	-0.247	0.327	-0.031	-0.026	-0.382	-0.257	0.107	-0.362	**0.641**	0.451	-0.380	-0.370
NO_3-N	0.181	0.211	-0.025	-0.060	-0.146	0.160	**-0.823**	0.333	-0.232	-0.074	-0.013	0.023	0.307	-0.054	-0.231	0.121	-0.159	**-0.803**	-0.097	-0.198
NH_4-N	**0.796**	0.241	-0.217	0.122	-0.445	0.085	**-0.580**	0.192	-0.442	0.262	0.375	0.056	0.300	0.065	0.295	0.414	**-0.571**	**-0.601**	**-0.573**	-0.572
Cl^-	-0.062	-0.281	-0.259	-0.163	0.225	0.036	0.545	0.011	-0.359	0.345	-0.055	0.022	-0.412	-0.347	0.065	-0.378	**0.583**	0.412	-0.443	-0.429
Fe	-0.190	-0.282	-0.186	-0.248	0.276	0.000	**0.696**	0.013	-0.130	0.116	-0.020	0.112	-0.297	-0.158	0.179	-0.247	**0.634**	0.623	-0.294	-0.242
Na^+	-0.172	-0.362	-0.288	-0.270	0.294	-0.026	**0.625**	0.065	-0.247	0.185	-0.034	0.102	-0.344	-0.240	0.174	-0.289	**0.684**	0.485	-0.333	-0.307
TSS	-0.237	-0.194	-0.104	-0.249	0.156	0.156	**0.691**	-0.077	-0.112	0.181	-0.147	0.112	-0.284	-0.176	0.033	-0.227	**0.573**	**0.697**	-0.279	-0.233

"...Все-таки желательно, гражданин артист, чтобы вы незамедлително разоблачили бы перед зрителями технику ваших фокусов...

...Пардон! - отозвался Фагот, - я извиняюсь, здесь разоблачать нечего, все ясно. "

Mikhail Bulgakov
Master and Margarita

CHAPTER 3

Multivariate analysis: bracken as a factor in study streams

3 Chapter objective

This chapter assesses the importance of bracken as a factor influencing the composition of invertebrate and diatom communities in the upland study streams in North Wales. The following topics are introduced: the use of macroinvertebrates and diatoms as indicators of stress and changing conditions; factors influencing their community composition with particular reference to British upland streams, and acidification as the main such factor in the UK.

3.1 Introduction

3.1.1 Macroinvertebrates as bioindicators

Macroivertebrates have been used extensively as an indicator of environmental conditions in running waters (Metcalfe-Smith, 1992). Unlike chemical data, which are often instantaneous in nature and, therefore, require multiple sampling, biological communities integrate environmental conditions over time (De Pauw & Vanhooren, 1983). Benthic invertebrate communities are especially suitable due to their abundance in aquatic habitats (Reynoldson, 1984), ease of collection (Plafkin *et al.*, 1989), established taxonomy (Reynoldson, 1984), quick reaction and differential sensitivity to different pollutants (Cook, 1976), long life spans, which provide a record of environmental quality (Pratt & Coler, 1976) and finally their heterogeneity, with numerous taxa and trophic levels (France, 1990).

3.1.2 Environmental factors affecting invertebrates in running waters

Explaining distribution and abundance of biota is a fundamental goal of ecology. Running waters are an ideal ecosystem where this question can be studied. Processes that structure communities in running waters often occur on spatial and temporal scales, which allow direct observation and manipulation; streams provide ecologists with natural replication on several scales, from different sized substratum particles to repeating pool-riffle sequences. Lotic ecosystems undergo environmental changes with a frequency and intensity that few others can match (Power *et al.*, 1988).

The question of what determines the structure of invertebrate communities has been answered insofar as that the main factors have been identified. These can be divided into abiotic and biotic. The former include physiochemical characteristics of running waters and their catchments, the latter are biological interactions between organisms, such as competition and predation. Naturally, both biotic and abiotic factors interact to a great degree and the effects of individual factors are difficult to isolate. Studies on invertebrate communities can be split into three categories. Firstly, there are studies which assess the importance of physiochemical variables. In these studies communities of several neighbouring streams are compared and related to differences in environmental variables. Studies of the second category, those that focus on ecological interactions between species (McAuliffe, 1984a,b; Kohler, 1992; Hart, 1985; Kuhara *et al.*, 1999), are usually restricted to one stream. This is also true for the third category of studies, which assess the importance of disturbance (McAuliffe, 1984a, Death, 1996; Matthaei & Townsend, 2000) in allowing coexistence between species. Therefore, the question of scales emerges - the effects of physiochemical variables are investigated on a higher scale, with replication, as several neighbouring streams are studied, whereas the studies on the effects of biotic factors are usually restricted to individual catchments. Extrapolating these findings across latitudes may not, therefore, be appropriate. Streams are found in all climatic and geographical zones. Local geology, topography and climate determine the structure of the stream, its water chemistry, and hydrological regime, setting up the overriding factors, which determine which organisms can colonize and persist in this particular environment and which can not. Biological interactions between the organisms that are adapted to each particular stream environment are undoubtedly important and most streams are likely to have both biotic and abiotic controls (McAuliffe, 1984; Power *et al.*, 1988) with the importance of each varying between streams.

This study is concerned with the influence of environmental factors with bracken as one of the potential factors. Out of a great number of physiochemical variables studied, several are regarded as being of primary importance. These include substratum, temperature, current and several water chemistry variables. Substratum is effectively the habitat of invertebrates in the stream, and its properties, such as size (Cummins & Lauf, 1969, Erman & Erman, 1984, Minshall, 1984), stability (Allan, 1995) and heterogeneity (Erman & Erman, 1984), have all been shown to affect the invertebrate community composition. Other environmental factors such as current (Hynes, 1970), oxygen concentration (Allan, 1995) and the amount of organic matter (Rabeni & Minshall, 1977) are also affected by substratum. This highlights its importance and also brings to attention the difficulties in attributing causality in streams to a single factor. Temperature affects growth rates (Sutcliffe *et al.*, 1981, Sweeny, 1984) and life cycles of stream organisms (Hynes, 1970) as well as the productivity of the entire stream (Hynes, 1970). Current, like substratum, is the immediate 'environment' and an adaptive force for stream biota. Water velocity influences the composition of substratum particles, delivery of gases and food (Allan, 1995). Upland streams have distinct physiochemical characteristics, reviewed in a previous chapter, and will thus have a specific hierarchy of physiochemical factors affecting the macroinvertebrate community.

3.1.3 Abiotic factors in upland streams

A great deal of research effort has been allocated to identification of physiochemical factors, which influence taxonomic and trophic structure of macroinvertebrate communities in upland streams in the U.K. The aim was to separate the effects of factors which are consequences of acidification, from those of naturally occurring ones. A number of physiochemical factors are measured for several neighbouring streams, macroinvertebrate species assemblages from these streams are ordinated using multivariate software packages, and/or classified by TWINSPAN (Hill, 1979a), a polythetic divisive clustering software package. Species assemblages are then related to the physiochemcal factors.

It has repeatedly been demonstrated that aluminium concentrations, total hardness and pH, all acidity-related water chemistry variables, are the most important in structuring the macroinvertebrate community composition in upland streams (Jenkins *et al.*, 1984; Rutt *et al.*, 1989, 1990; Weatherly & Ormerod, 1990; Rundle *et al.*, 1992; Ormerod *et al.*, 1993). Acidity affected both species richness (Rundle et al., 1992; Ormerod et al., 1993) and trophic structure of the community (Rundle *et al.*, 1992). Another group of factors measured as part of this survey approach, and found to be explaining a significant amount of variation in community composition, were the so-called 'location' variables: linkage, width, depth, slope (Rutt *et al.*, 1990; Rundle *et al.*, 1992; Ormerod *et al.*, 1993;), altitude (Jenkins *et al.*, 1984; Rutt *et al.*, 1990; Ormerod *et al.*, 1993), catchment area (Rutt *et al.*, 1990). Their significance in influencing the community structure is expressed when the streams selected for a survey vary considerably in altitude and size.

3.1.4 Acidification

Streams are considered acidified when their mean annual pH is below 5.7-5.4 (Sutcliffe & Hildrew, 1989). Acidification of upland streams in the UK was brought about by slow natural loss of base cations during post-glacial soil formation, then accelerated by land-use change, and finally, in the past 150 years, the rates of acidification have soared due to atmospheric deposition of sulphur and nitrogen after the Industrial Revolution (Emmett *et al.*, 1984).

Freshwater acidification occurs when an increase in anion concentration is not accompanied by an increase in buffering cations (Emmett *et al.*, 1984). The incoming anions may originate from the atmosphere or are generated from within the soil. Acidification is a complex process involving the chemistry of precipitation water, catchment soils and hydrological pathways of the catchment. Several water chemistry features are affected by acidifiction. These include concentrations of H^+, sulphate, nitrate, and several metals, such as aluminium, manganese and zinc (Ormerod & Jenkins, 1994). Acidity of freshwaters, and hence concentrations of these chemical determinands, is in a state of flux. During rainstorms pH can plunge drastically, accompanied by a rise in concentrations of aluminium, for several hours. After a snowmelt this acid episode can last for several weeks; a forest clearance or a drought can produce an acid episode of several months. Finally, chronic acidification of a stream can last for years. Chronically acidified streams, however, still undergo acid episodes, which are thought to be important physiological stressors to the biota (Weatherley & Ormerod, 1991).

Acidification has significantly altered the fauna of upland streams. Ephemeroptera, caddisflies of families Philopotamidae and Hydropsychidae, crustaceans and molluscs disappear or become scarce with decreased pH. Stoneflies, however, are often abundant (Sutcliffe & Hildrew, 1989).

The effect of acidity on benthic invertebrates is expressed through direct lethal physiological effects and via undermining of the food-base. Uptake of ions by insects is disrupted under acid conditions. Sodium is lost from the body and insufficient calcium is taken up from the surrounding waters (Økland & Økland, 1986). The loss of these ions from blood and tissues is lethal. Aquatic insects are thought to vary in their susceptibility to ion loss (Willoughby & Mappin, 1988). Macrocrustaceans and molluscs are even more susceptible than insects, especially during moult when permeability to water is increased. The evidence that pH exerts part of its effect on stream invertebrates via the food supply is nowhere near as strong as that for the direct physiological effects, but is, nevertheless, suggestive. Two potential mechanisms have been put forward: influence on the decomposition process of allochthonous organic matter, and lowering of food quality for grazers. Decomposition of tree leaf litter slows down with decreased pH (Minshall & Minshall, 1978; Hildrew *et al.*, 1984), as microbial populations responsible for leaf break-down are reduced (Sutcliffe & Hildrew, 1989).

Much attention has been paid to the structure of periphyton in acidic streams. Lack of grazing invertebrates has been attributed to low nutritional quality of biofim (structureless film with few living components) under acidic conditions (Townsend *et al.*, 1983; Winterbourn *et al.*, 1992). The effect of pH on the biofilm, however, is still unclear, the results of both experimental studies (Maurice *et al.*, 1987; Planas *et al.*, 1989) and stream surveys (Mulholland *et al.*, 1986; Collier & Winterbourn, 1990; Kinross *et al.*, 1992) are equivocal, and no clear relationship between pH and biofilm structure has been found.

In addition, to macroinvertebrates, acidification, both directly and indirectly, adversely affects fish (Turpenny *et al.*, 1987), bryophytes (Ormerod *et al.*, 1987), birds (Ormerod *et al.*, 1985), and mammals (Mason & Macdonald, 1987) of running waters in the uplands. This, in turn, exerts effects on macroinvertebrate communities via trophic relationships.

3.1.5 Diatoms

The algal component of the biofilm is one of the main sources of autochthonous primary production in streams and rivers (Hildrew, 1992), and in upland streams where there are very few rooted angiosperms and associated algal epiphytes, the importance of periphyton in primary production is potentially even greater. Algal grazing, therefore, is a very important link in the food webs of streams (Lamberti & Moore, 1984; Hildrew, 1992). There is ample evidence of bottom - up resource limitation of grazers in streams (McAuliffe, 1984b; Lamberti *et al.*, 1987; Lamberti *et* *al.*, 1989; Winterbourn *et al.*, 1992; Ledger & Hildrew, 2000). If bracken does have an effect on periphyton in upland streams, it would be likely to manifest itself further up the trophic web. Grazing invertebrates, such as mayflies, elminthid beetles and some caddis would be affected even if there are no direct toxic effects from bracken on invertebrates themselves.

Diatoms are usually the dominant component of periphyton (Patrick, 1961; Chudyba, 1965; Moore, 1972), other components being green algae, cyanobacteria and fungi. Many factors are known to affect the composition of periphyton, such as irradiance (Hill *et al.*, 1995), nutrient concentrations (Elwood *et al.*, 1981), temperature (Darley, 1982), substratum composition (Bott, 1982) and disturbance (Peterson & Stevenson, 1992; Biggs & Thomsen, 1995). Variation in these factors leads to high temporal and spatial variation in the biomass and structure of biofilm (Ledger & Hildrew, 1998).

Diatom assemblages undergo changes in species composition in response to a range of stressors, and are, therefore considered excellent bioindicators, having been used as indicators of metal stress (Foster, 1982) and nutrient enrichment (Marcus, 1980; Kelly, 2002). Numerous studies have demonstrated the responsiveness of diatom communities to environmental conditions (Vanlandingham, 1976; Jones, 1978; Stevenson, 1984; Chessman, 1986; Wunsam *et al.*, 2002). In the U.K. diatoms have been instrumental in the study of acidification. Their microfossils preserved in lake sediments have been successfully used in reconstruction of pH changes (Battarbee, 1984). Even though these paleolimnological studies usually involve lakes, the species cross-over between upland lenthic and lotic systems allows the use of the same classification of acid tolerance for upland streams.

Battarbee (1984) describes the progressive acidification in terms of diatom community change as follows: as pH declines to 5.5-5.0 alkaliphilous species such as *Achnanthes microcephala*, *Fragilaria virescens*, *Cymbella gracilis* and *Nitzschia perminuta*, become replaced by acidophilous *Tabellaria flocculosa*, *Frustulia rhomboides*, and several species of the genus *Eunotia*. Continuing acidification below 5.5 leads to an increase in the acidobiontic taxa, which are only found in waters with pH below 5.5. These include *Tabellaria binalis*, *Eunotia exigua* and *Anomoeneis serians*. This pattern of species change is dependent on the initial composition of the diatom community and is also obscured by the presence of generalist taxa such as *Achnanthes minutissima*.

Little is known about the physiological basis of the effect of acidity on diatoms. Reduction of the amount of biologically available orthophosphate due to its precipitation with increased dissolved aluminium concentrations (Hsu & Rennie, 1962) is thought to be the main reason (Smith, 1990b). Other possible factors include changes in nutrient chemistry with acidification (Nalewajko & O'Mahoney, 1988), and increases in concentrations of metals (Dillon *et al.*, 1988). Generally, little is known about the factors which are important in diatom ecology (Round, 1990).

The potential toxicity of bracken to diatoms (aquatic algae and plants in general) has never been studied. The question of bracken phytotoxicity has been addressed in the studies on allelopathic effects of bracken on terrestrial species (Glass, 1975; Gliessman, 1976; Gliessman & Miller, 1972; Nava *et al.*, 1987; Taylor & Thompson, 1990; Dolling *et al.*, 1994). The validity and ecological relevance of these are discussed in the Introduction to Chapter 4. Some of the reported effects (after administration of bracken extracts) included inhibition of radicle growth of several cultivated species, such as maize (*Zea mays*) and peanut (*Arachis hypogaea*) (Nava *et al.*, 1987), and inhibition of germination and seedling growth of aspen (*Populus tremula*) and Scots pine (*Pinus sylvestris*) (Dolling *et al.*, 1994).

3.2 Methods

Invertebrates were kick-sampled using a pond net with 250μm mesh. Sampling was carried out in May and July of 1999. At each site two riffles and two pools were sampled. The duration of each kick-sample was 2 minutes. The riffles and pools were chosen at random from all the habitats of that type. Only habitats of length greater than 2 metres were included in the sampling programme. A pilot sampling run had shown 2 metres to be the approximate length of a channel sampled in two minutes. After each kick sample the length and width (three replicates) of the area sampled was measured in order to obtain an estimate of the number of invertebrates per unit area of the stream bed. The samples were preserved in 90% ethanol solution. In the laboratory the samples were put through a series of sieves with meshes of 2, 1, 0.5

and 0.25 mm. The contents of sieves were transferred into plastic trays from which the invertebrates were then sorted and separated to the lowest possible taxonomic level. Chironomids and water mites were counted without sorting. Invertebrates were then identified using appropriate keys (Davies, 1968; Edington & Hildrew, 1981; Elliott *et al.*, 1988; Hynes, 1977; Wallace *et al.*, 1990). The individuals of the species belonging to the shredder and grazer trophic guilds were counted. The information on the trophic status of species was taken from a selection of sources (Wetzel, 1983; Groom & Hildrew, 1989; Allan, 1995; Winterbourn & Hildrew, 1985). Invertebrate densities were calculated as the number of individuals per square metre by dividing the number of individuals by the area of the streambed for each sample taken.

Diatoms were sampled in spring summer and autumn of 1999 at the same time as the invertebrates. Three riffles, selected at random from all riffles were sampled from each study stream. See the methods section of chapter 2 for the methodology used in diatom sampling. The slides of diatom samples were prepared as follows. Ten milliliters of sample was centrifuged for three minutes at 1500 r.p.m. The supernatant was poured off and 2.5 ml of distilled water and the same amount of concentrated nitric acid were added to the tube. The tubes were kept for one hour in boiling water in a fume cupboard. The volume was then topped up to 12.5 ml, the contents of the tube mixed and centrifuged again. After centrifuging, pH was tested with Litmus paper. If found to be below 6, the supernatant was discarded and the volume made up to 12.5 ml. This procedure was repeated until the pH of the supernatant was between 6 and 7. The supernatant was then removed with a pipette, 5 ml of distilled water added to the tube, the contents mixed and transferred into scintillation vials. Cover slips were put on a glass grid and 0.5 ml of each sample was placed into the middle of each cover slip. Samples that contained a very high density of diatoms were diluted prior to mounting. The samples were left overnight to dry. Glass slides were labeled and a small drop of naphrax added to the centre of each with a glass rod. The cover slips were then placed on the slides and put onto a warm hotplate to dry off the solvent and removed when rapid bubbling had stopped. The samples were then left to harden for 24 hours. Diatoms were identified using Krammer & Lange-Bertalot (1991) and their abundance expressed as thousands per cm^2.

For methods of measuring physiochemical factors see the method section of Chapter 2. The environmental and species data were analyzed on the multivariate statistics software package CANOCO for Windows (ter Braak, 1988), using direct gradient analysis methods: redundancy analysis (RDA, used when the response to the environmental variable is a linear) or canonical correspondence analysis (CCA, used when the response is unimodal). See Table 7 for the list of environmental variables used in the analyses. RDA was used if the lengths of the ordination axes were less than 3 standard deviations in the detrended correspondence analysis (DCA) which was performed on the species data prior to CCA or RDA. The data from each of the sampling occasions (May and July for invertebrates, May, July and August for diatoms) were analyzed separately for both invertebrates and diatom data. The percentage of shredders of the total number of invertebrates in spring and summer was tested for correlations with the bracken characteristics of each study stream: frond density, frond height and area under bracken. The same procedure was also carried out separately on the ten Berwyn streams.

Table 7. Environmental variables used in direct gradient analysis of invertebrate and diatom data. Diatom data also included density of grazers.

Catchment-scale variables	Altitude	**Bracken**	Bracken frond density
	Stream length		Bracken frond length
	Catchment area		Bracken area
			Total bracken
Physical variables	Depth		
	Width	**Chemistry**	pH
	Moss cover		Ca^{+2}
	Riffle area %		Mg^{+2}
	Run area %		Cl^{-}
	Pool area %		Fe
	Cascade length %		Na^{+}
	Periphyton chlorophyll		Nitrate - N
	Shading		Ammonia - N
			Conductivity
Substratum	Stream substratum volume		Alkalinity
	Riffle substratum volume		Total suspended solids
	Pool substratum volume		
	Run substratum volume		

3.3 Results

3.3.1 Invertebrates

Invertebrate fauna of the study streams was typical of the uplands. See Table 8 for the list of taxa recorded in the study streams. The community was dominated by stoneflies (Plecoptera), with *Isoperla grammatica, Leuctra spp., Amphinemura sulcicollis, Protonemoura meyeri* and *Chloroperla torrentium* being the most common species. Another abundant group included beetles of the family Elminthidae, especially *Elmis aenea*. Caseless Trichoptera (caddisflies) were represented by several species of the genus *Rhyacophila*, and two species of *Plectrocnemia*: *P. conspersa* and *P. geniculata*. Other families, such as Hydropsychidae (*H. instabilis* and *H. siltalii*) and Philipotamidae (*P. montanus*) were very rare in the Berwyns, but common in the Clwyds. Cased caddis were predominantly of the Limnephilidae family. Three additional families: Goeridae, Odontoceridae and Glossosomatidae were found almost exclusively in the Clwydian streams. *Baetis* (largely *B. vernus*), *Rhithrogena semicolorata* and *Siphlonurus lacustris* comprised the mayfly fauna of the study streams. Their abundance was biased towards the Clwydian streams, and even though common in some Berwyn streams (Afon Llynor), they were extremely rare in others (Ceunant Coch). Presence of *Gammarus pulex*, gastropods (*Ancylus fluviatilis, Limnaea peregra* and *Valvata spp.*), and bivalves (*Pisidium*) in the Clwydian streams was another difference between the two groups of streams.

3.3.1.1 Redundancy analysis of Berwyn and Clwydian data

The spring (Fig.29) and summer (Fig.30) ordinations are very similar in the spread of species points on ordination space and also in terms of positions of species-points in relation to each other. The bulk of the species-points is found in the right half of the ordinations. The outermost species points are the same: the less acid-tolerant taxa (*Gammarus pulex*, ephemeropterans *Baetis* and *Siphlonurus lacustris*, molluscs *Ancylus fluviatilis* and *Limnaea peregra*) are on the right, the acid-tolerant caddis *Plectorcnemia conspersa* and plecopterans *Chloroperla torrentium* and *Nemoura cinerea* on the left. The vertical boundaries are set by *Leuctra*, *Protonemoura meyeri*, *Amphinemura sulcicollis* at one end, and *Bezzia* and *Hydropsyche siltali* at the other. See Table 9 for eigenvalues of the first two RDA axes.

Table 8. Invertebrate taxa recorded in the study streams.

Trichoptera caseless	Plectrocnemia geniculata
	P. conspersa
	Rhyacophila obliterata
	R. semicolorata
	R. munda
	R. dorsalis
	Hydropsyche siltalii
	H. instabilis
	Philopotamus montanus
Trichoptera cased	Agapetus fuscipes
	Chaetopteryx villosa
	Drusus annulatus
	Halesus digitatus
	Halesus radiatus
	Micropterna spp
	Melamopophylax mucoreus
	Odontocerum albicorne
	Potamophylax cingulatus
	Silo pallipes
	Caddis type 1 (unidentified)
	Caddis type 2 (unidentified)
Diptera	Bezzia
	Chironomidae
	Clinocera spp
	Dicranota spp
	Dixa spp
	Pedicia spp
	Pericoma spp
	Protosimulium averense
	Ptychoptera spp
	Simulium spp
	Stratiomyidae
	Tabanus
	Diptera types 1-11 (unidentified)
Oligochaeta	Oligochaeta
Collembola	Collembola
Hemiptera	Aphelocheirus aestivalis
	Corixa spp
Megaloptera	Sialis fuliginosa
Plecoptera	Amphinemura sulcicollis
	Brachyptera risi
	Chloroperla torrentium
	Dinocras cephalotes
	Diura bicaudata
	Isoperla grammatica
	Leuctra spp
	Nemoura cinerea
	Nemoura spp (other)
	Nemurella pictetii
	Protonemoura meyeri
Ephemeroptera	Baetis spp.
	Rhithrogena semicolorata
	Siphlonurus lacustris
Coleoptera	Agabus chalconatus
	Anacaena globulus
	Colymbetes spp
	Elmis aenea
	Esolus parallelepipedus
	Haliplus spp
	Helodidae
	Helophorus flavipes
	Hydraena nigrita
	Limnius volkmari
	Oulemnius spp
	Coleoptera type 1 (unident)
Odonata	Cordulegaster boltonii
Hydracarina	Hydracarina
Crustacea	Copepoda
	Gammarus pulex
Ostracoda	Ostracoda
Hirudinia	Helobdella stagnalis
Mollusca	Ancylus fluviatilis
	Limnaea peregra
	Pisidium spp
	Valvata spp

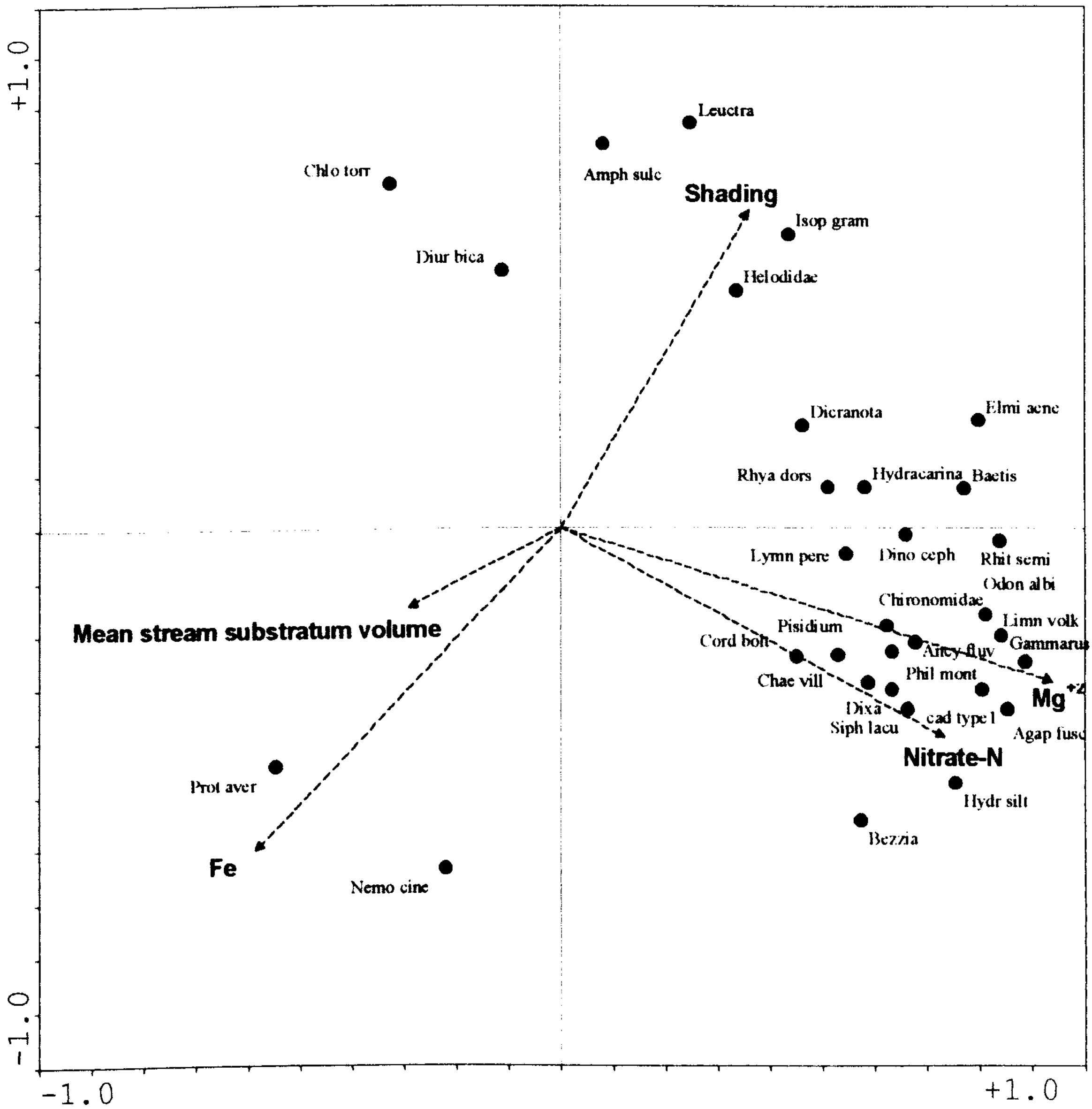


Fig.29 Redundancy analysis ordination of spring invertebrate data from all study streams. Note: the direction of the 'shading' arrow corresponds to a decrease in the shading of a stream. Shredders in red.

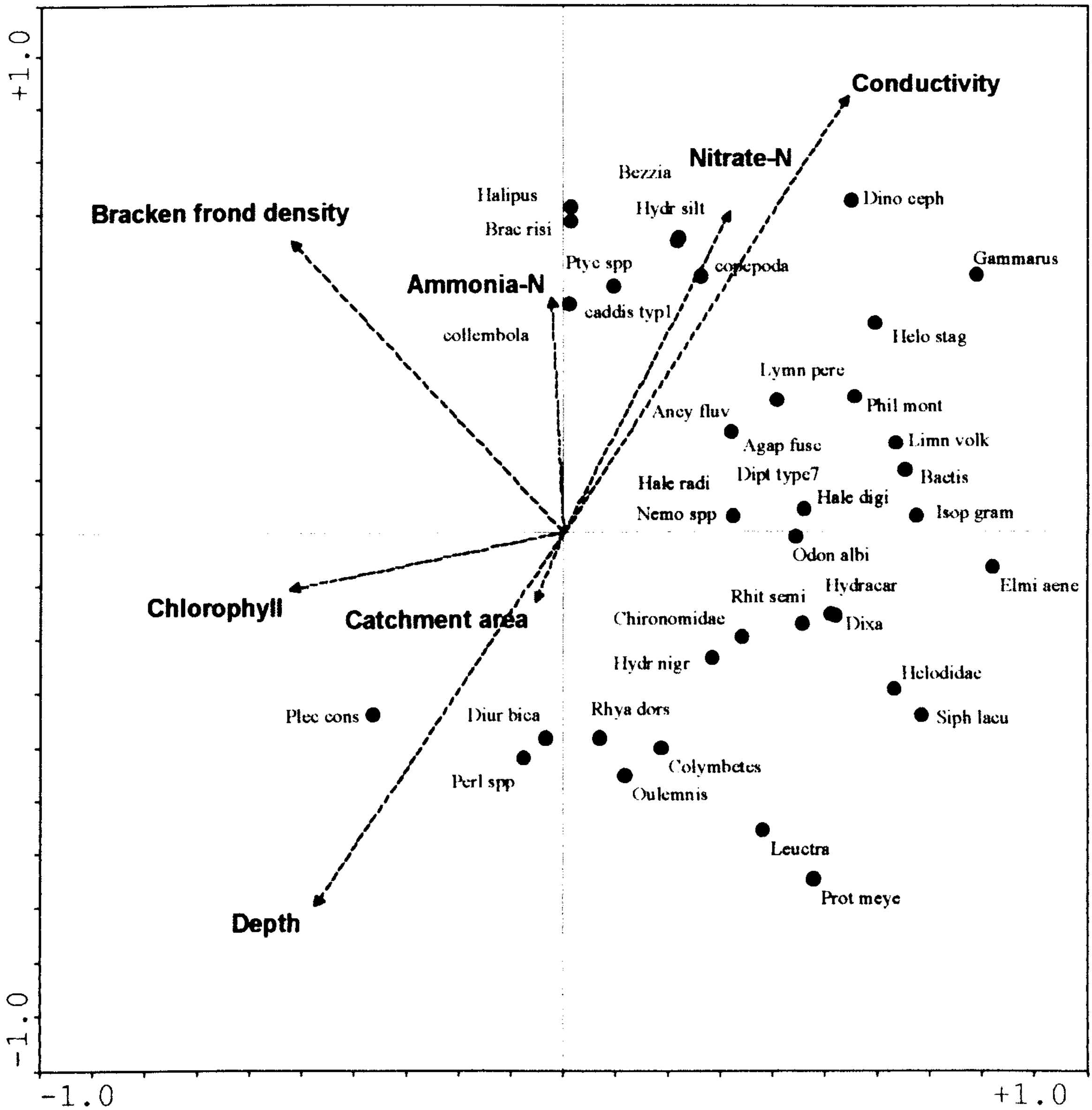


Fig.30 Redundancy analysis ordination of summer invertebrate data from all study streams. Shredders in red.

Table 9. Eigenvalues of the first two RDA axes of invertebrate data.

Ordination	*All streams*		*Berwyn*	
	Axis 1	Axis 2	Axis 1	Axis 2
Spring	0.345	0.147	0.348	0.068
Summer	0.234	0.125	0.225	0.101

The arrows representing environmental variables form the arrangement of two axes perpendicular to each other. The first is a gradient of chemical variables: Mg^{+2} and nitrate-N in spring and conductivity and nitrate-N in summer increase in one direction. Physical variables: catchment area and depth increase in the opposite direction to these chemical variables on the summer ordination. This chemical / physical axis is at the right angle to bracken frond density on the summer ordination. Frond density is absent on the spring ordination, and its position taken up by the Fe arrow.

The proximity of species-points to variables' arrows indicates the degree of preference by species for these variables. The tightest clustering is around the chemical variables, especially those associated with acidity. In contrast, no species-points are associated with bracken frond density, and only two with Fe. The clustering of species points around the axes of Mg^{+2} and nitrate-N in spring is much greater than around nitrate-N and conductivity on the summer ordination. This suggests the chemical variables have greater importance in spring than in summer.

3.3.1.2 Berwyn invertebrate data

The arrangement of species points was largely the same as in the ordinations of the combined Berwyn and Clwydian data. No bracken variables emerged as significant. See Table 9 for eigenvalues of the first two RDA axes. The spring ordination (Fig.31) showed a chemical gradient not seen on other ordinations, with Fe and conductivity increasing in one direction, and Mg^{+2} in the opposite. Iron was also perfectly correlated with the first RDA axis. This again confirmed the importance of chemistry in spring and illustrated the separation of Berwyn sites into iron-rich ones (Nant Cwm Pydew, Nant Sgrin, which also had the longest stretches of cascades), and less acidic iron-poor ones (Clochnant, Nant Esgeiriau). Ammonia again was a significant variable, and was closely co-aligned with the second RDA axis.

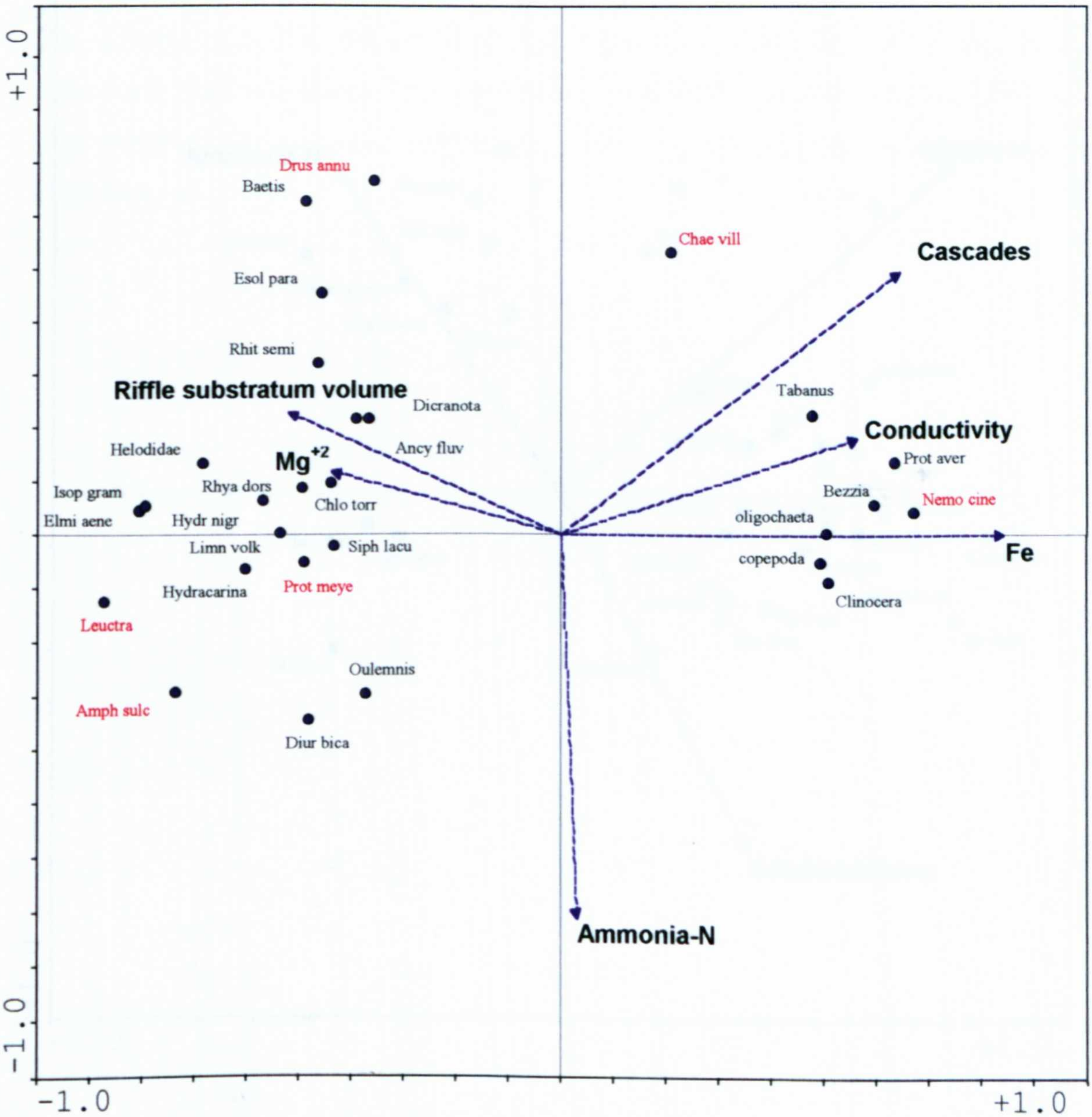


Fig.31 Redundancy analysis ordination of Berwyn spring invertebrate data. Shredders in red.

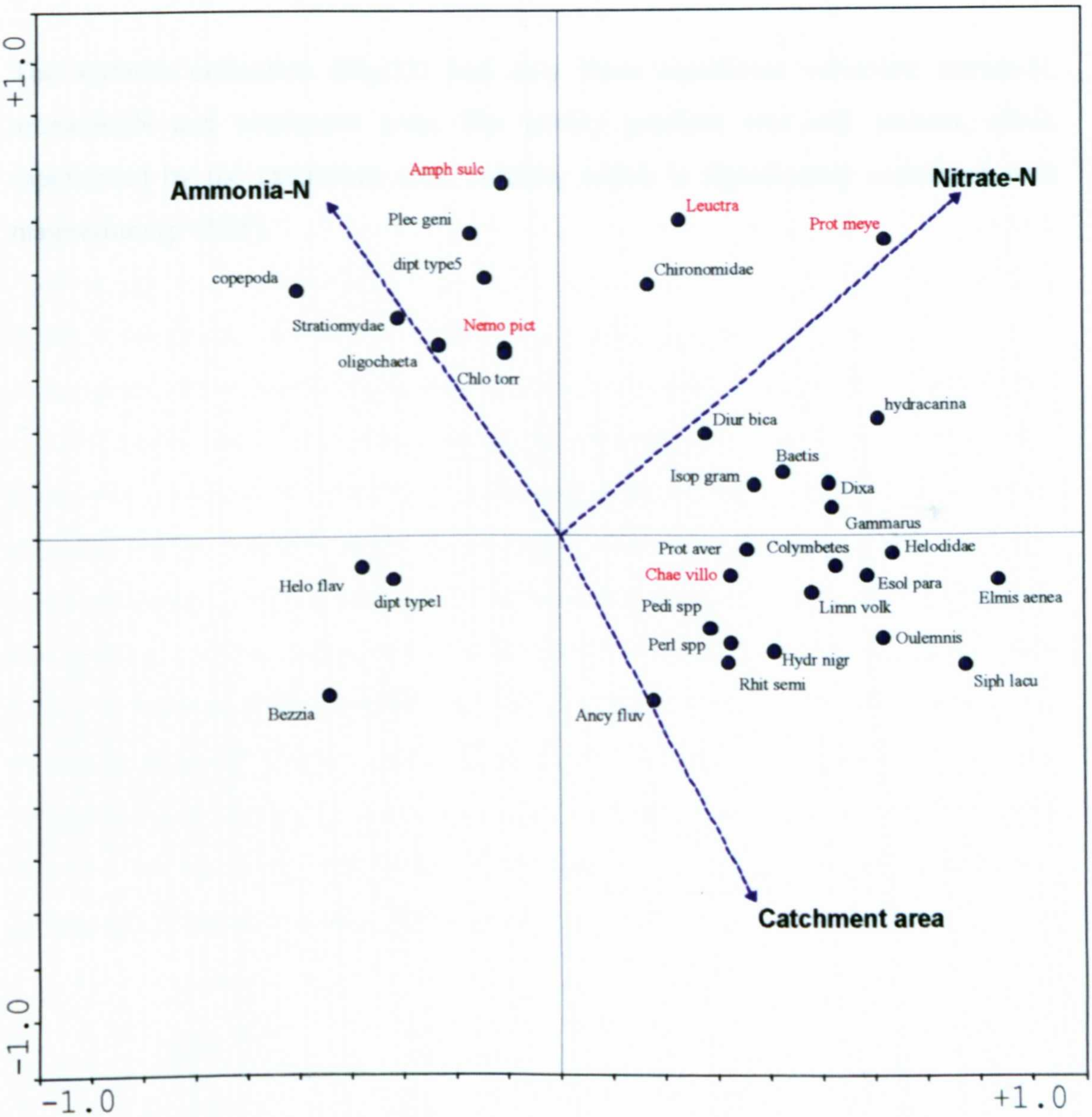


Fig.32 Redundancy analysis ordination of Berwyn summer invertebrate data.

The summer ordination (Fig.32) had only three significant variables: nitrate-N, ammonia-N and catchment area. The acidity gradient was still present, albeit represented by the catchment area variable, which is significantly correlated with magnesium ($p<0.05$).

3.3.1.3 Shredders and bracken

In the Berwyns the most abundant shredders were the nemourid stoneflies *Amphinemura sulcicollis*, *Protonemoura meyeri* and the genus *Leuctra*. In Cwm Pen Llydan, where half of all invertebrates were shredders, 98% of these were either *Leuctra spp*. or *A. sulcicollis*. *Leuctra* was one of the most numerous shredder taxa during both seasons, whereas *A. sulcicollis* was most common in spring and *P.meyeri* in summer. Less numerous, but common were cased caddis *Chaeotopteryx villosa* and *Drusus annulatus*. Two other nemourid plecopterans: *Nemoura cinerea* and *Nemurella pictetii* were present in small numbers as were cased caddis *Halesus digitatus* and *H. radiatus*. In the Upper and Lower streams in the Clwyds shredders were represented overwhelmingly (95%) by *Gammarus pulex*, the rest being mainly the caddis *C. villosa* with very few plecopterans. The Reservoir stream had a more balanced make up of its shredder taxa with *Leuctra*, *P. meyeri* and *Gammarus* found in similar numbers. The positions on the ordination (Fig.30 summer, all streams) of the points corresponding to shredder taxa are associated with either low bracken frond density: *Amphinemura sulcicollis*, *Protonemoura meyeri*, *Leuctra*, or show no preference: *Gammarus pulex*, *Halesus radiatus*.

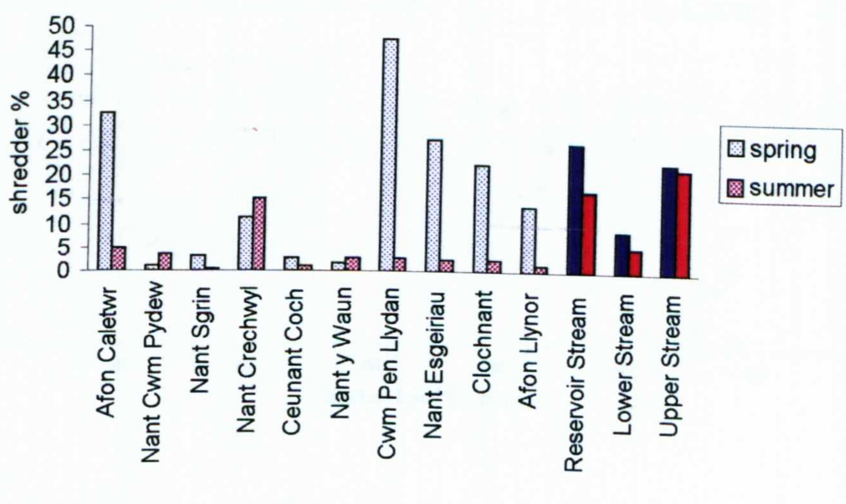


Fig.33 Shredders as the percentage of the total number of invertebrates. Clwydian streams highlighted.

Percentage of shredders varied from almost 50% of all invertebrates in Cwm Pen Llydan, in spring to less than 2% in Nant Sgrin (Fig.33). In the Berwyn the spring

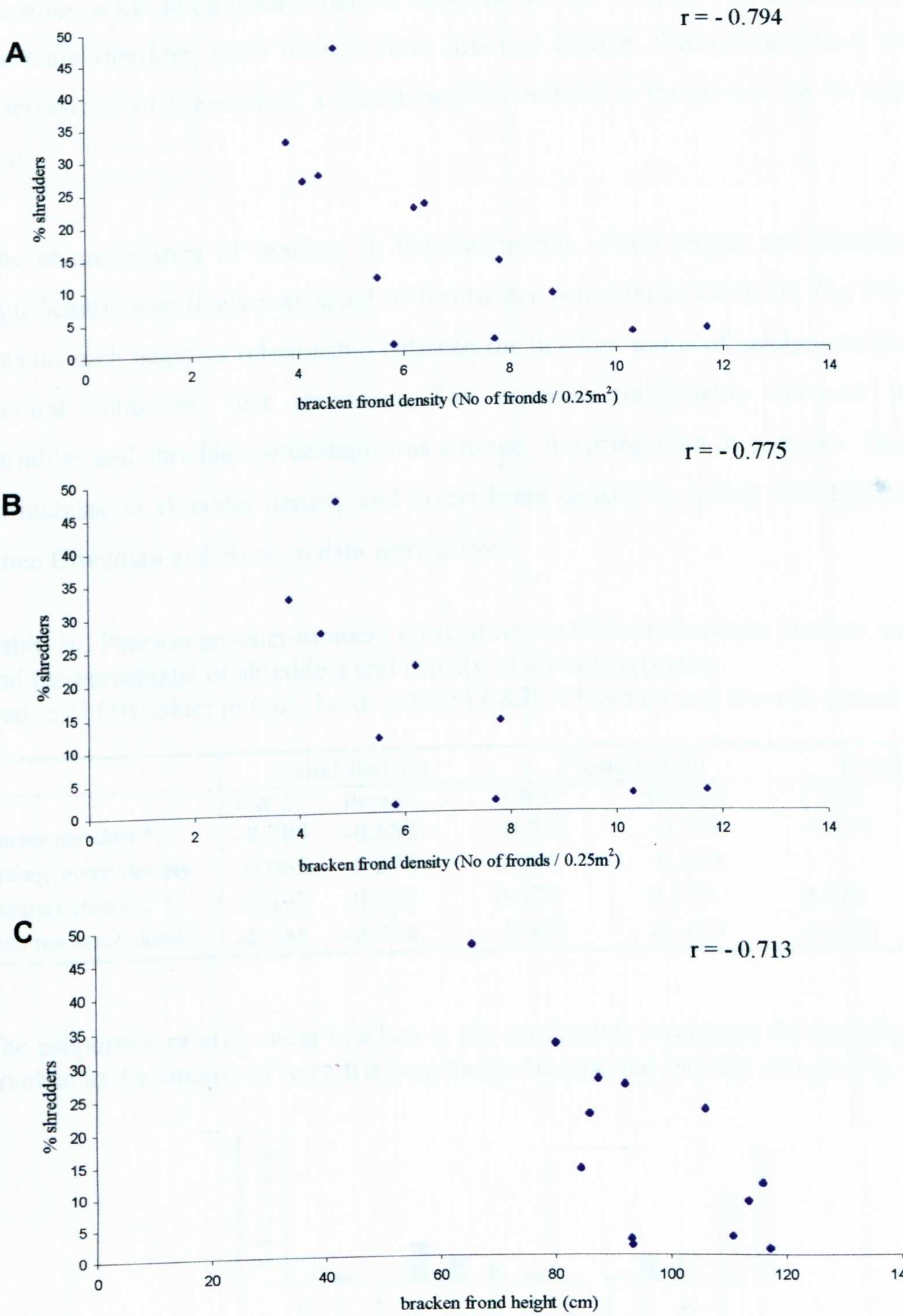


Fig.34 Relationship between the percentage of shredders in the invertebrate communities in May and: A. bracken frond density; B. bracken frond density in the Berwyn; C. bracken frond length.

densities were much greater than in summer. In the Clwyds, where *Gammarus* was the main shredder, there was no clear seasonal change. Plecopterans have seasonal emergence, but *Gammarus*, a crustacean, is confined to the stream for its entire life span.

The characteristics of bracken in the catchments, frond length and density, were significantly negatively correlated with shredder percentage (Table 10; Fig.34). There was no such negative relationship between the bracken status of catchments (i.e. total amount contained) and shredders. The negative relationship between bracken variables and shredder percentage was stronger in spring than in summer. There was an increase in shredder density and invertebrate density in spring with bracken area when Clwydian and Berwyn data were pooled.

Table 10. Pearson product-moment correlation coefficients between bracken variables and the percentage of shredders and density of all invertebrates.
(red: $p<0.001$; blue: $p<0.01$; bold: $p<0.05$) C&B: Clwydian and Berwyn stream data.

	Frond density		Frond height		Bracken area	
	C & B	Berwyn	C & B	Berwyn	C & B	Berwyn
Spring shredder %	**-0.709**	**-0.685**	**-0.732**	**-0.790**	-0.194	-0.462
Spring invert density	-0.065	-0.374	0.291	-0.244	**0.782**	-0.149
Summer shredder %	-0.392	-0.433	0.379	0.379	**0.620**	0.240
Summer invert density	**-0.748**	**-0.738**	-0.454	-0.497	-0.249	-0.236

The proportion of area under bracken in the catchments represents the contribution of bracken to the amount of terrestrial vegetation transported into the stream (Fig.35)

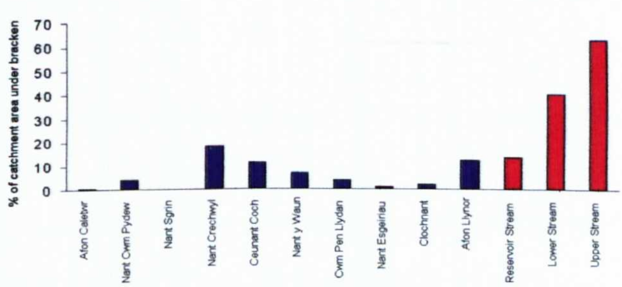


Fig.35 Percentage of the catchment area under bracken. Clwydian streams in red.

There was a significant positive correlation between percentage of catchment area under bracken, and summer shredder percentage (Table 11).

Table 11. Pearson product moment coefficients of correlations between percentage of catchment area under bracken and the shredder variables ($p<0.01$**; $p<0.05$*).

	Clwyd & Berwyn	Berwyn
Spring shredder %	-0.057	-0.337
Summer shredder %	**0.692****	**0.599***

3.3.2 Diatom data

The diatom communities of the study streams were very similar, with almost every recorded species found in all study streams (See Table 12 for the list of taxa recorded in the study streams). *Achnanthes minutissima*, *Gomphonema gracile*, *Eunotia praeerupta* and *Fragilaria capucina* were the most common species. In the Clwyds these were joined by *Cocconeis placentula*. Both alkaliphilious and acidophilous taxa were present. The former included *Fragilaria viriscens, Cymbella gracilis, Epithimia spp.*, the latter *Tabellaria flocculosa, Frustulia rhomboides, Navicula radiosa,* and the genus *Eunotia*. Acidobiontic *Eunotia exigua var compacta* was found, but was very rare. Acidophilous taxa were co-dominant with generalist species (*Achnanthes minutissima, Gomphonema gracile*) in all streams regardless of pH (Table 13).

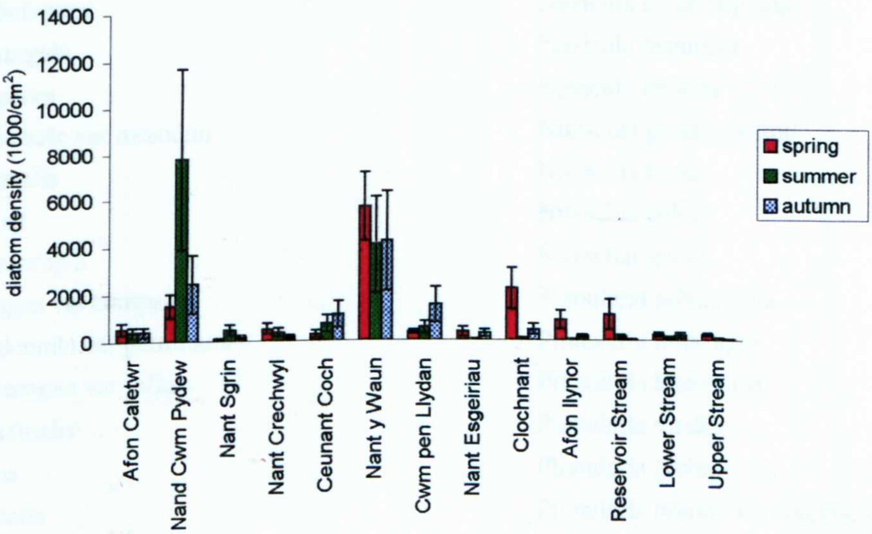


Fig.36 Densities of diatoms on the substrata of the study streams. Mean ± SEM, n = 3.

The streams differed, however, in overall diatom densities (Fig.36). The substratum in the Clwydian streams supported fewer diatoms than that in most of the streams in the Berwyn. Some Berwyn streams (Nant Esgeiriau) were, however, comparable with the Clwydian streams during all three seasons, others (Clochnant, summer) only during a single season. Diatom densities did not show the same seasonal peak in all streams, although in seven out of thirteen streams the highest densities were in spring. These

Table 12. Diatom taxa recorded in the study streams.

Achnanthes conspicua var brevistriata
Achnanthes lanceolata var rostrata
Achnanthes grimmei
Achnanthes lanceolata
Achnanthes minutissima
Achnanthes exilis
Achnanthes grimmei var
Amphora pediculus
Anomoeoneis brachysira
Campylodiscus clypeus var bicostatum
Ceratoneis arcus
Cocconeis placentula
Cyclotella comta
Cyclotella meneghiniana
Cymbella graciis
Cymbella helvetica
Cymbella turgida
Cymbella parva
Diatoma hiemale var mesodon
Diploneis ovalis
Epithemia sp
Eunotia praerupta
Eunotia exigua var compacta
Eunotia tridentula var perminuta
Eunotia interrupta var inflata
Eunotia pectinalis
Eunotia faba
Eunotia lunaris
Eunotia valida
Eunotia parallela
Fragilaria virescens
Fragilaria virescens var elliptica
Fragilaria capucina
Fragilaria pinnata
Fragilaria pinnata var
Fragilaria harrissonii
Frustulia rhomboides
Gomphonema gracile
Gomphonema subtile
Gomphonema acuminatum var trigonocephala
Gomphonema gracile var lanceolata
Gomphonema olivaceum
Gomphonema augur
Meridion circulare
Meridion circulare var constricta
Navicula laterostriata
Navicula bacillum
Navicula minima
Navicula cryptocephala
Navicula radiosa
Navicula hasta
Navicula schonfeldii
Navicula capitata var hungarica
Navicula minima
Navicula amphicephala
Navicula disjuncta
Navicula rotaena
Nitzschia gandersheimii
Nitzschia acuta
Nitzschia palea
Nitzschia gisela
Pinnularia subcapitata
Pinnularia interrupta
Pinnularia leptosoma
Pinnularia viridis
Pinnularia gibba
Pinnularia braunii var amphicephala
Pinnularia viridis
Pinnularia intermedia
Rhoicosphenia curvata
Rhopalodia gibba
Surirella linearis
Surirella ovata
Synedra affinis
Synedra ulna
Synedra rumpens var familiaris
Synedra vaucheriae
Tabellaria flocculosa

Table13. Diatom communities of the study streams with reference to acidity. Clwydian streams' names' in bold.

Stream	Peak density (1000/cm^2)	Peak season	Mean annual pH	Dominant species
Afon Caletwr	547	Spring	5.24	*Anomoeoneis brachysira* *Eunotia praeerupta* *Eunotia pectinalis*
Nant Cwm Pydew	7783	Summer	5.69	*Eunotia praeerupta* *Achnanthes minutissima* *Gomphonema gracile*
Nant Sgrin	471	Summer	5.29	*Eunotia praeerupta* *Achnanthes minutissima* *Fragilaria capucina*
Nant Crechwyl	513	Spring	5.82	*Achnanthes minutissima* *Eunotia praeerupta* *Diatoma hiemale var mesodon*
Ceunant Coch	1110	Autumn	5.11	*Achnanthes minutissima* *Eunotia praeerupta* *Achnanthes lanceolata*
Nant y Waun	5666	Spring	5.85	*Eunotia praeerupta* *Frustulia rhomboides* *Gomphonema gracile*
Cwm pen Llydan	1489	Autumn	5.75	*Achnanthes minutissima* *Eunotia faba* *Eunotia praeerupta*
Nant Esgeiriau	346	Spring	5.67	*Achnanthes minutissima* *Eunotia praeerupta* *Achnanthes lanceolata*
Clochnant	2163	Spring	6.26	*Achnanthes minutissima* *Diatoma hiemale var mesodon* *Gomphonema gracile*
Afon Llynor	865	Spring	5.98	*Achnanthes minutissima* *Diatoma hiemale var mesodon* *Gomphonema gracile*
Reservoir Stream	1078	Spring	6.40	*Achnanthes minutissima* *Fragilaria capucina* *Diatoma hiemale var mesodon*
Lower Stream	243	Spring	6.89	*Cocconeis placentula* *Achnanthes minutissima* *Achnanthes conspicua var brevistrata*
Upper Stream	249	Spring	6.98	*Achnanthes minutissima* *Cocconeis placentula* *Diatoma hiemale var mesodon*

included all of the less acidic Berwyn streams, such as Nant Esgeiriau, Clochnant and Afon Llynor and the three Clwydian streams.

3.3.2.1 Direct gradient analysis

Unlike invertebrate analyses, where the arrangement of species-points on the ordinations and the nature of significant environmental variables remained largely unchanged with season, the ordinations of diatom data showed marked seasonal changes. Seasonally equivalent ordinations of total data (Clwyd and Berwyn) and Berwyn streams, were, however, similar.

3.3.2.1.1 Spring

The arrangement of the arrows of the significant variables in spring (Fig.37) forms two axes. The first axis is an altitude/chemistry gradient. Conditions of lower altitude are on the top left, with greater grazer density and and higher pH. Grazer species included: all Ephemeroptera, all elminthid beetles, trichopterans *Silo pallipes*, *Odontocerum albicorne*, *Agapetus fuscipes*; plecopteran *Brachyptera risi*; molluscs *Ancylus fluviatilis*, *Limnaea peregra* and *Valvata spp.* Moving diagonally across to the bottom right of the ordination, the conditions change to those of higher altitude: lower pH, larger substratum particles and greater amount of periphyton chlorophyll. The second axis is of lesser importance than the first, and reflects hydrological differences between streams. Fast-flow conditions, where riffles are common (bottom left of the ordination), change to slower flow, as the percentage of runs increases. Variation explained by the first two RDA axes was 39%, with 23.4% explained by axis 1 and 15.6% by axis 2.

In the Berwyn streams (Fig.38) all variables, apart from chlorophyll, formed the altitude/chemistry axis, which almost coincided with the second RDA axis. High pH, and greater concentration of calcium at lower altitude are opposed by higher concentrations of sodium and iron as altitude increases. The positions of species-points on total streams and Berwyn ordinations are the same both in their locations on the ordination space and relative to other species. The acidophilous taxa (*Tabellaria flocculosa*, *Eunotia*, *Frustulia rhomboides*) are on the far right of both ordinations, and the more generalist and cirumneutral taxa, such as *Achnanthes minutissima* and

Fragilaria at the top. Variation explained by the first two RDA axes stood at 40.1%, with axis 1 accounting for 22.9% and axis 2 for 17.2%.

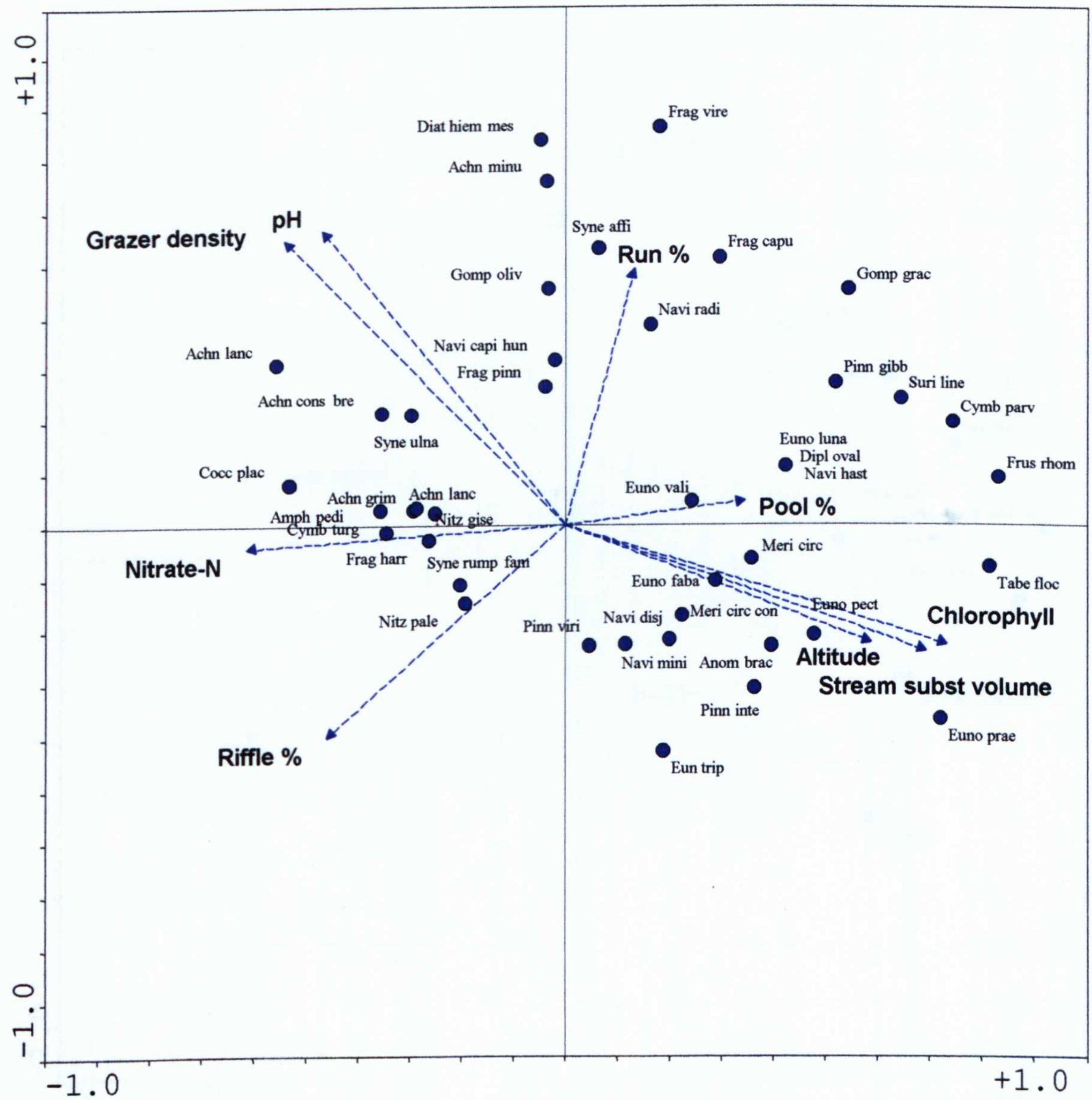


Fig.37 RDA ordination of spring diatom data.

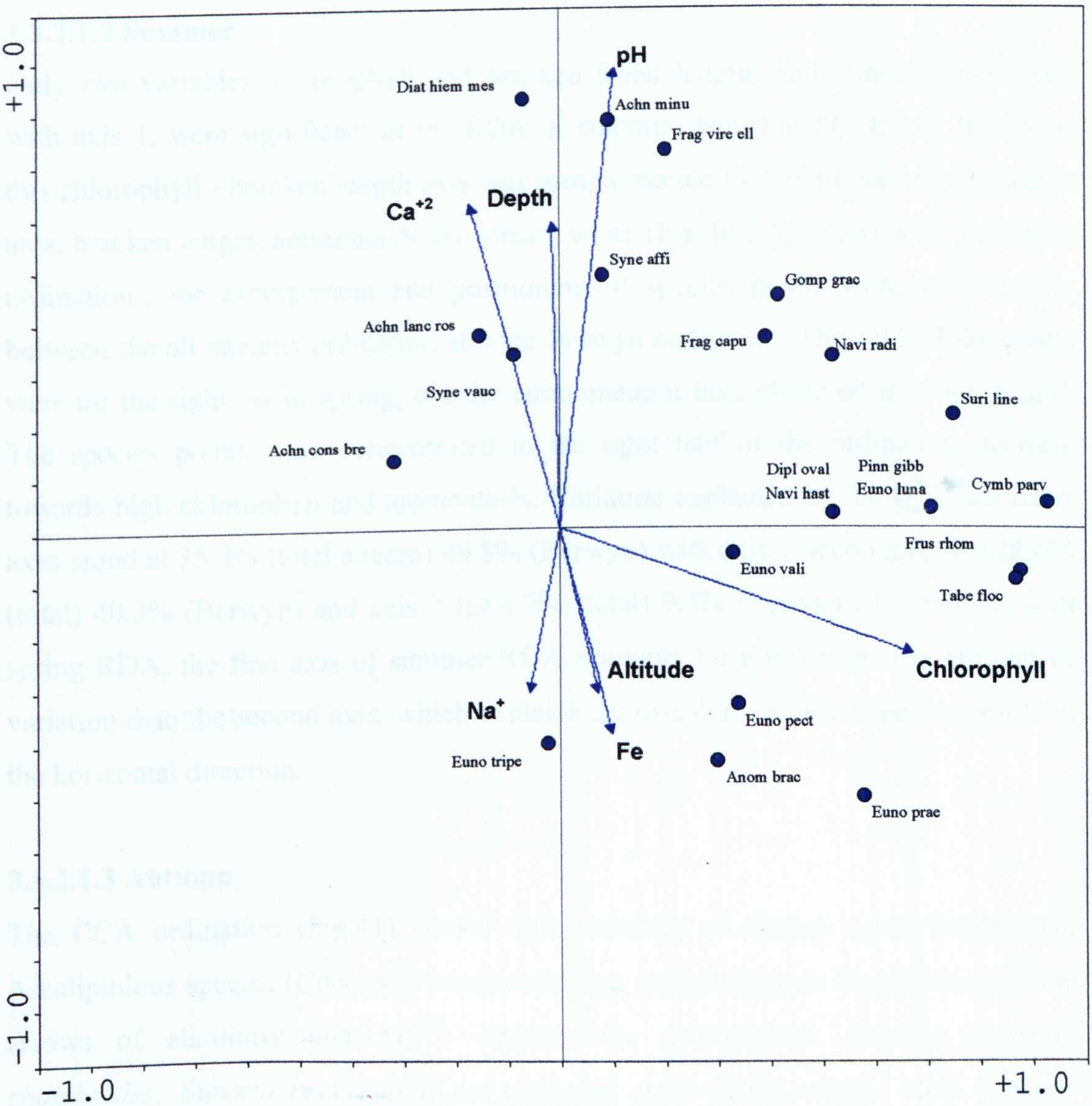


Fig.38 RDA ordination of Berwyn spring diatom data.

3.3.2.1.2 Summer

Only two variables, chlorophyll and bracken frond length, both closely co-aligned with axis 1, were significant in the RDA of summer data (Fig.39). In the Berwyns, this chlorophyll - bracken length axis was complemented by less important catchment area, bracken length, ammonia-N and stream width (Fig.40). Again, as with the spring ordinations, the arrangement and positioning of species points were very similar between the all streams ordination and the Berwyn ordination. The acidophilous taxa were on the right, as in spring, but the circumneutral taxa clustered near the origin. The species points were concentrated in the right half of the ordination, tending towards high chlorophyll and ammonia-N. Variation explained by the first two RDA axes stood at 35.3% (total stream) 49.8% (Berwyn) with axis 1 accounting for 28.6% (total) 40.3% (Berwyn) and axis 2 for 6.7% (total) 9.4% (Berwyn). In contrast with spring RDA, the first axis of summer RDA accounts for a much greater amount of variation than the second axis, which explains the distribution of the species points in the horizontal direction.

3.3.2.1.3 Autumn

The CCA ordination (Fig.41) shows four variables of almost equal importance. Alkaliphilous species (*Cocconeis placentula*), are pulled along in the direction of the arrows of alkalinity and Mg^{+2}, whereas the acidophilous species (*Frustula rhomboides, Eunotia praeerupta*) are clustered close to the origin. Most species-points show either no preference in terms of chlorophyll levels or are associated with low chlorophyll conditions (*Navicula minima, Pinnularia subcapitata*). The Berwyn ordination (Fig.42) consists entirely of variables that describe physical (percentage of riffles, width, depth, catchment area) and biological (chlorophyll) characteristics of streams. The amount of variation explained by the first two CCA axes was 65.9% (total stream) and 57.3% (Berwyn) with axes 1 accounting for 40.7% (total) and 37.6% (Berwyn) and axes 2 for 25.6% (total) and 19.7% (Berwyn).

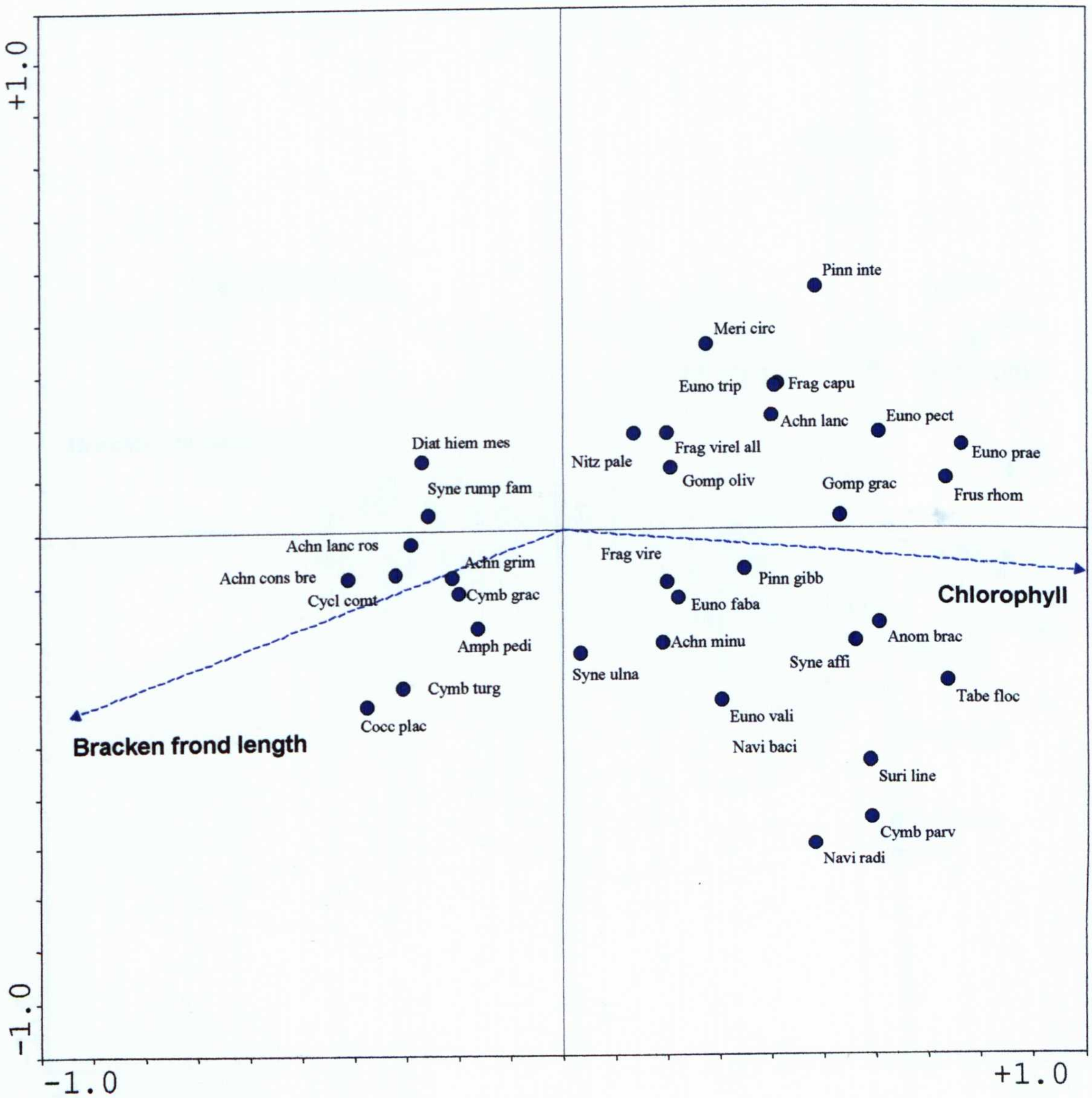


Fig.39 RDA ordination of summer diatom data.

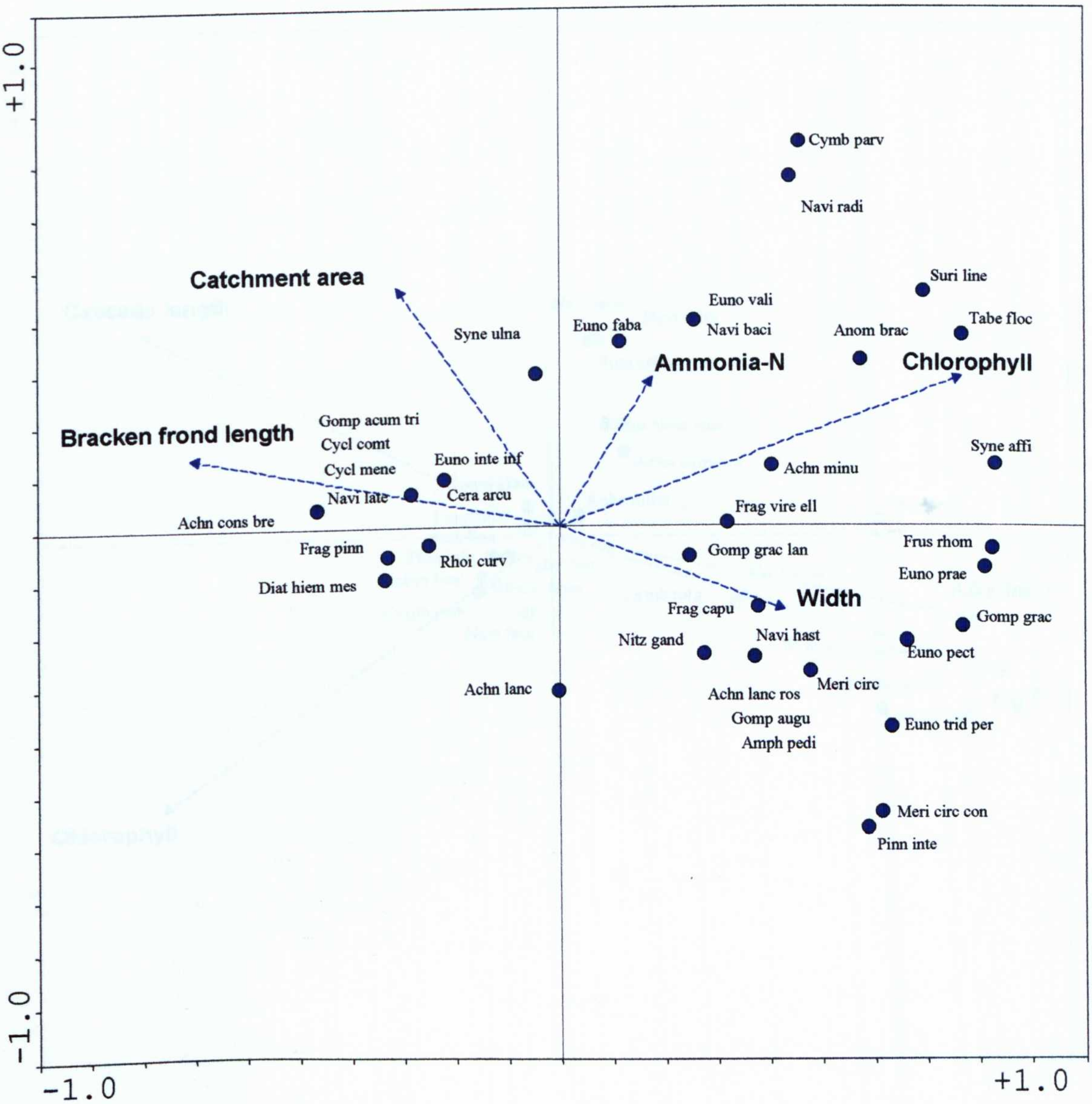


Fig.40 RDA ordination of Berwyn summer diatom data.

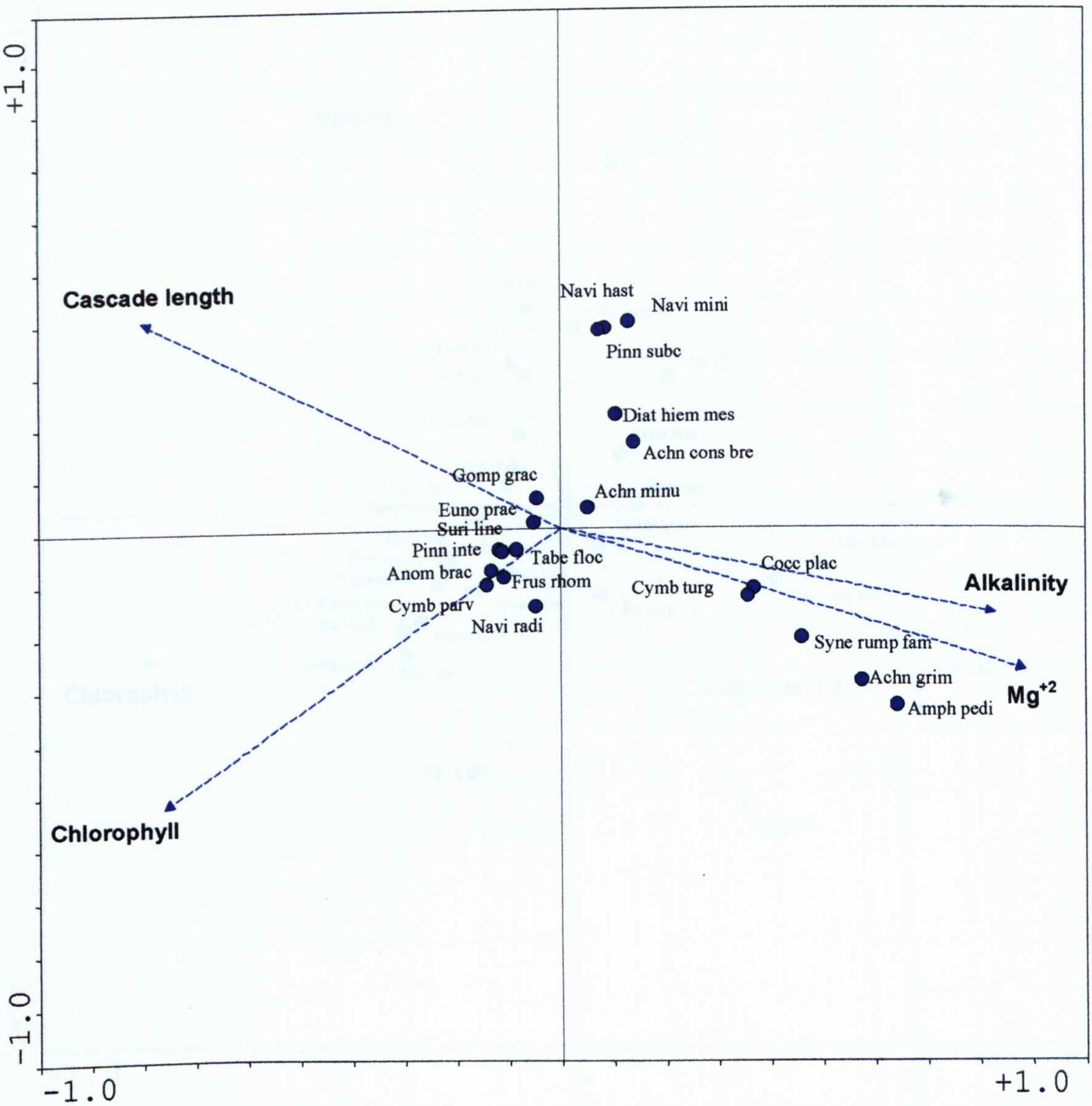


Fig.41 CCA ordination of autumn diatom data.

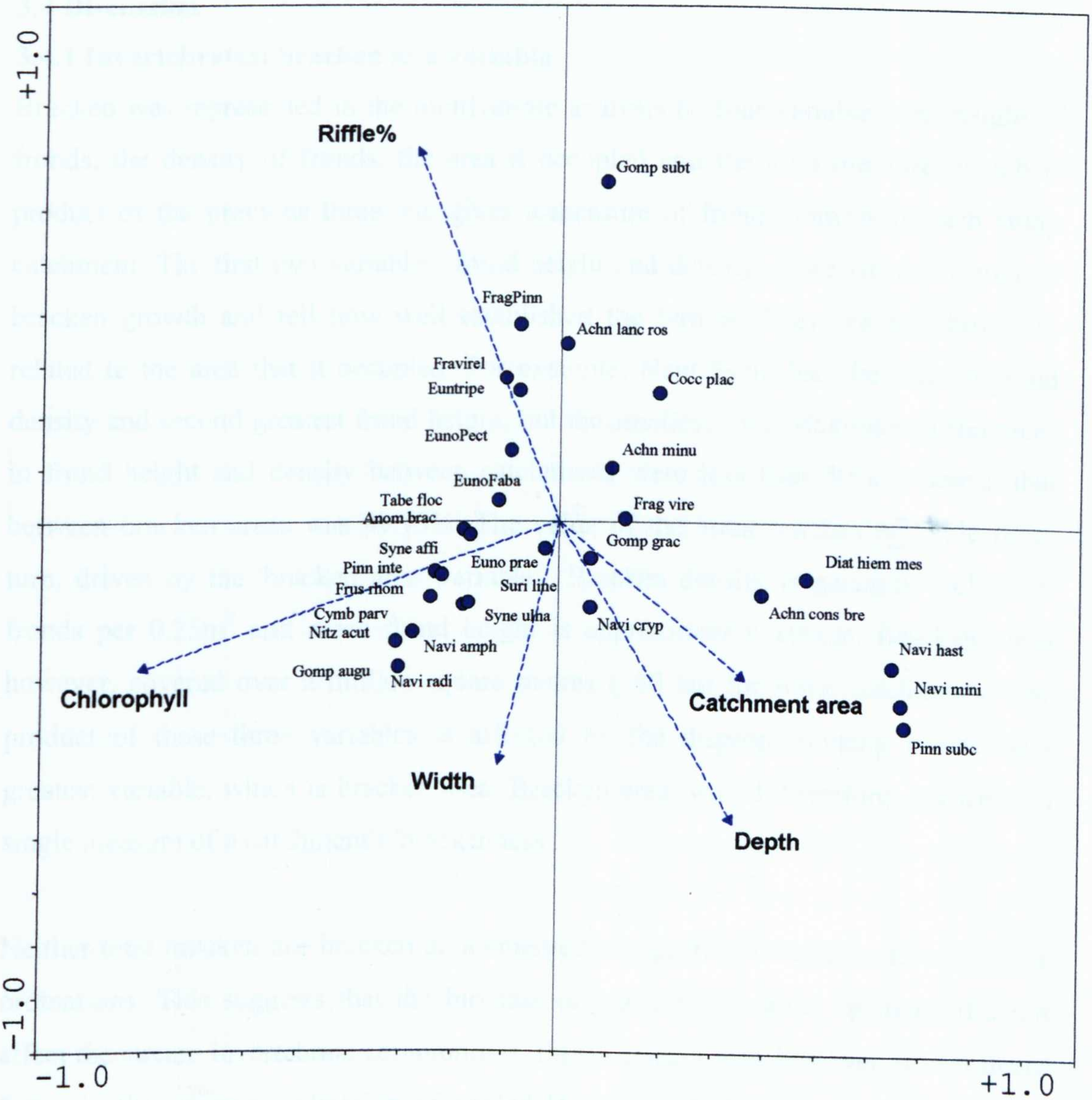


Fig.42 CCA ordination of Berwyn autumn diatom data.

3.4 Discussion

3.4.1 Invertebrates: bracken as a variable

Bracken was represented in the multivariate analysis by four variables: the height of fronds, the density of fronds, the area it occupied and the total measure, which is product of the previous three and gives a measure of frond biomass in each study catchment. The first two variables, frond height and density, represent the vigour of bracken growth and tell how well established the fern is. They are not, however, related to the area that it occupied. For example, Nant Sgrin had the highest frond density and second greatest frond height, but the smallest area. Maximum differences in frond height and density between catchments were less than 50%, whereas that between bracken areas was 99.93%. The value of the 'total bracken' variable is, in turn, driven by the 'bracken area' variable. Bracken density is generally below 10 fronds per $0.25m^2$ and mean frond height is approximately 100cm. Bracken areas however, covered over a million square metres (100 ha) for some catchments. The product of these three variables is affected by the disproportionally numerically greatest variable, which is bracken area. Bracken area, would, therefore, suffice as a single measure of a catchment's 'brackenness'.

Neither total bracken nor bracken area emerged as significant variables in any of the ordinations. This suggests that the biomass of bracken contained upstream did not affect the stream invertebrate communities. Frond density was, however, a significant factor in the summer ordination of pooled Berwyn and Clwydian data. A possible explanation for this seasonal character is that toxic effects are not exerted until bracken reaches its growth peak in summer. However, at the time of sampling (May) bracken fronds were fully developed, well past the crozier stage, and the canopy of bracken stands was almost closed. Also, the concentration of many toxins, including ptaquiloside, is at its peak in younger fronds (Cooper-Driver, 1977; Alonso-Amelot *et al.*, 1992; Potter & Pitman, 2000), and there is a decline in total bracken toxin content and potential as the growth season progresses, culminating in inert senescent fronds (Potter & Pitman, 2000). It is, therefore, more reasonable to suppose there being a greater toxin transport from fronds into the stream in spring than in summer, especially when precipitation is greater in spring.

It is more credible to regard frond density as a surrogate for some factor not been included in the survey. As already mentioned in Chapter 2 discussion, the soil of the study catchments is variable, and includes several soil types, the proportions of which vary among the catchments. From visual observation each catchment had peaty, iron-rich, humic gley soils, which are waterlogged and thus cannot support bracken. If the area under dry podzolic soils, which are preferred by bracken, is surrounded by gleys, bracken in these catchments is limited in space into which it can expand once it has outcompeted other plant species in these islands of podzols. The bracken stands in most of study streams are over 50 years old (A. Price, personal communication, 1999), and have had ample time to exert their dominance. In the catchments of Nant Sgrin, Nant Cwm Pydew, Nant y Waun and Ceunant Coch all non-waterlogged area at suitable altitude is under very dense high bracken. These catchments also have extensive gley soils. It appears that where bracken density and frond height are the greatest, the proportion of gleys is the highest, as the boxed-in bracken rhizome may be maximizing the density of fronds it produces in the limited area available.

Presence of gleys in the catchment influences the chemistry and physical properties of stream water. Streams draining gley-rich catchments tend to be more turbid, with more solids suspended in the water (Chalupa, 1966), high concentrations of iron (Shapiro, 1966), and lower pH due to presence of organic humic acids (Collier *et al.*, 1990; Winterbourn & Collier, 1987; Otto & Svensson, 1983). Such brown-water running waters are products of natural acidification and are found across the globe (Otto & Svensson, 1983). In the UK uplands they are an example of a naturally acidic system acidified even further by anthropogenic activity. Unfortunately, the resolution of the available soil maps of the area was not sufficient to calculate the area under gleys in each of the study catchments, and include it in the analysis as an independent variable.

Table 14. Pearson product-moment coefficients of correlations between bracken frond density and bracken frond height in the catchments and the levels of iron and total suspended solids in the stream water. * signifies $p<0.05$.

	Clwyd & Berwyn		*Berwyn*	
	Pt density	Pt height	Pt density	Pt height
Fe	0.481	0.441	0.641*	0.607*
TSS	0.321	0.592*	0.633*	0.656*

Table 14 shows that density and height of fronds did increase with the amount of Fe and TSS in the stream water, and that these correlations were statistically significant, especially in the Berwyn streams. The Clwydian catchments of the Upper and Lower streams lack gley soils and the only restrictions on the expansion of bracken are altitude at the top and improved grassland/farmland at the bottom of the valleys. High bracken density is due to the fact that bracken stands in the Clwyds are very old and the last control measures (localized cutting) were carried out more than 30 years ago (H.Davies, Clwyd AONB Warden, 2002 personal communication). Also, more favourable climatic conditions in the Clwyds owing to their lower altitude may permit more vigorous bracken growth than is possible in the higher Berwyn catchments. The inclusion of the high bracken density, but low gley Clwydian streams, therefore, breaks down the correlation between these two variables.

Another confirmation that bracken density acts as a surrogate for chemical/physical water variable or variables, comes from the arrangement of species-points around the variable arrows on the RDA ordinations. *Bezzia* and *Nemoura cinerea* are positioned counter to *Leuctra*, *Amphinemura sulcicollis* and *Protonemoura meyeri* on all ordinations. In the summer all streams ordination (Fig.30), the only one where bracken frond density is selected as significant, the arrow for this variable coincides with this species arrangement, with the latter group associated with low bracken density. These same species are also associated with the Fe arrow when it is present on the spring ordinations. The ordination of the spring Berwyn data is where the differences due to gley - related water chemistry are best highlighted. Iron is the single most important variable, and is perfectly correlated with the first RDA axis. This splits the species points into two groups, with those tolerant of very acidic, high Fe conditions (*Nemoura cinerea*, *Protosimulium averense*, *Bezzia*) clearly separated from the rest.

Studies on brown water streams have concentrated primarily on acidity of humic and fulvic acids as the main deleterious factor to the biota of such streams (Saber & Dunson, 1987; Winterbourn & Collier, 1987, Collier *et al.*, 1990). Unknown additional factors have also been suggested, albeit for amphibians (Saber & Dunson, 1987). Elevated concentration of iron is likely to be one of the additional sources of toxicity, which seems especially possible in the Berwyn streams. Mean annual Fe concentrations of Nant Cwm Pydew (1.83mg/l) and Nant Sgrin (2.67 mg/l) are comparable with those from New Zealand streams contaminated by acid mine drainage (Winterbourn *et al.*, 2000). The peak Fe concentrations in the same two study streams in July 1999 of 9.66 and 4.65 mg/l respectively exceed those of most of the contaminated New Zealand streams, whereas there were no anthropogenic sources of iron in the Bewryn. High turbidity also resulted in precipitation of organic matter, especially in Nant Sgrin and Nant y Waun, which may smother slow-moving and sedentary animals and possibly interfere with the delicate gill structure of mayflies.

3.4.2 Shredders

The negative correlations between shredder abundance and bracken frond density and height were highly significant. However, as has already been discussed, the measure of bracken biomass is the real measure of the amount of bracken available to shredders, and it is did not show the same relationship with shredder variables. This again brings to attention the relationship between bracken density and height and soil/water quality. As frond density and height are correlated with TSS and especially Fe, the relationship between density and length of bracken and shredder abundance is an artefact of the causative relationship between Fe concentrations and other characteristics of humic waters, and shredders.

The most common shredder species in the Berwyn were stoneflies *Leuctra*, *Amphinemura sulcicollis* and *Protonemoura meyeri*, with over 95% of all shredders belonging to these species in most streams. These were the species consistently associated with the lowest concentrations of Fe and lowest frond density on the RDA ordinations (Figs.29-32). These were also the most numerous taxa overall: in Cwm Pen Llydan in spring *A. meyeri* and *Leuctra* accounted for 47% of all invertebrates. A decline in invertebrate density, which occurs with humic conditions, therefore, disproportionally affects the most abundant taxa. The decline of shredders with increased bracken density, therefore, is probably due to a combination of their natural dominance of non-humic acid streams and sensitivity to humic conditions by the main taxa. Caddis *Halesus* and plecopteran *Nemoura cinerea*, which are very rare in iron-poor streams, are the chief shredders in humic streams, but their numbers are over a hundred times lower compared with those of *Leuctra*, *A. sulcicollis* and *P. meyeri* in non-humic streams. Tolerance of humic conditions probably enables them to survive while in humic streams, whereas in non-humic streams they may be outcompeted by other shredders.

Positive correlation of shredder percentage with bracken area when Clwydian and Berwyn stream data were analyzed together is due to high abundance of *Gammarus* in the Clwyd, where the areas under bracken are the greatest. It also explains the highly significant increase in invertebrate density with bracken area in spring, as *Gammarus* is one of the most numerous taxa in the Clwyd, accounting for example for over 20% of all invertebrates in spring in the Upper stream.

Increase in shredder percentage with increasing percentage of catchment area under bracken in summer (Table 11) is probably the result of a parallel increase in pH. In the combined Clwyd and Berwyn dataset percentage of area under bracken is correlated with bracken area ($p<0.001$), which, in turn, is correlated with pH ($p<0.01$). The same correlation, however, was also significant in the Berwyn where the correlation between pH and bracken area does not exist. The graph of this relationship (Fig.43), however, shows that this weakly significant correlation is largely the result of the outlier (Nant Crechwyl) extending the gradient, whereas the rest of the points do not show any clear relationship between shredder abundance and bracken dominance in the Berwyn catchments.

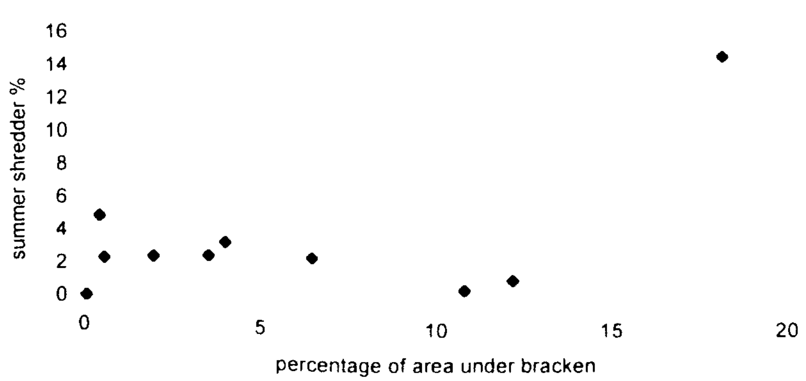


Fig.43 Relationship between the percentage of the catchment area under bracken in the Berwyn and the percentage of shredders in summer. The outlier on the far right is Nant Crechwyl.

3.4.3 Diatoms

As in the invertebrate analysis the measure of total bracken biomass did not emerge as significant in any ordinations suggesting no effect of bracken on the diatoms in the study streams. Bracken frond length, was, however, significant, and similarly to bracken frond density in the invertebrate analysis, it was significant in summer only.

Bracken frond length is significantly positively correlated with the concentrations of Fe and TSS, it would, therefore, be reasonable to draw another analogy with the results of the analysis of invertebrate data and consider frond length to be reflecting the effect of chemical/physical characteristics of humic streams. However, the species associated with the frond length arrows are not the same as those associated with the Fe arrow on the spring Berwyn ordination. In the spring Berwyn RDA ordination the Fe arrow was closely aligned with that of pH, and the associated taxa were three species of *Eunotia* and *Anomoeoneis brachysira*, all typical of acidic conditions. In both summer ordinations, however, these species points were aligned in the direction opposite to that of the bracken frond length.

Species associated with high frond length were the least abundant ones, such as *Cyclotella comta*, *C. meneghiana* and *Navicula laterostriata*. All three were recorded in very low numbers from a single riffle in Cwm Pen Llydan, the catchment of which, incidentally, has the shortest bracken fronds of all study streams. Other species close to the frond length arrow are also rare and found primarily in the less acidic Berwyn streams (Nant Esgeiriau, Cwm Pen Llydan) which also had the shortest bracken fronds. On the RDA ordination of Berwyn and Clwydian streams alkaliphilous *Cymbella gracilis* and *Cocconeis placentula* were also associated with high frond density.

In the Berwyn, frond length is significantly positively correlated with conductivity ($p<0.02$), which itself is highly positively correlated with concentrations of Cl^-, Fe, Na^+ and TSS ($p<0.001$). The summer ordination distinguishes between acidic, high conductivity (due to Cl^-, Fe, Na^+) conditions on the right and more circumneutral, but ion-poor on the left. The same separation occurred on the invertebrate ordination of the Berwyn summer data. The main gradient, therefore, appears to be that of acidity and conductivity. The common acidophilous species are on the right, the rare and alkaliphilous species on the left with the generalists (*Achnanthes minutissima* and *Gomphonema gracile*) approximately in the middle. The frond length arrow in fact points in the direction opposite to what it is expected, which can't be explained with the variables measured as part of the survey. It still, however, likely that frond density is included due to the link between bracken vigour and humic conditions of the streams.

The grazer density arrow is closely co-aligned with pH and its direction is directly opposite to that of the arrows for chlorophyll and altitude on the spring diatom data ordtination (Fig.37). The grazing taxa are all circumneutral species, ephemeropterans and molluscs, all of which are very abundant at the lower altitudes at which all the Clwydian streams and some of the less acidic Berwyn streams were situated . Low periphyton chlorophyll in these streams may be a consequence of higher grazing pressure, as grazers are known to reduce periphyton biomass (Lamberti *et al.*, 1989; Winterbourn *et al.*, 1992; Ledger & Hildrew, 2000). Differences between streams in other factors such as irradiance (Hill *et al.*, 1995), and substratum composition (Bott, 1982), however, would also contribute.

The most abundant species of diatoms are co-aligned with the chlorophyll arrow on all ordinations (Figs.37-42). These diatom species, being green plants, are likely to be responsible for this increase in chlorophyll, rather than displaying preference for existing high - chlorophyll conditions, which is another way of interpreting the ordination. It is not possible, however, to say what proportion of the total biomass of the periphyton the diatoms accounted for in the study streams, as the contribution of various components of periphyton was not quantified.

CHAPTER 4

Ecotoxicological experiment: the effect of bracken run-off on *Gammarus pulex*

4 Introduction

Bracken undoubtedly contains compounds that are toxic to biota, but many of the discovered toxic effects may be artefacts of experimental procedures. One issue is means of extraction, and the second one is the dosage given. The best demonstration of problems with the former are the studies of the allelopathic effects of bracken (Glass, 1975; Gliessman, 1976; Gliessman & Miller, 1972; Nava *et al.*, 1987; Taylor & Thomson 1990; Dolling *et al.*, 1994) for the following reasons.

Bracken extracts used in allelopathy experiments are supposed to imitate the rain run-off from bracken fronds and litter. In nature, when bracken fronds are exposed to rain, the water runs down from the canopy into the ground. The residence time of water on bracken fronds is therefore, likely to be quite short. In the experimental work, however, fronds were soaked in water for several hours and the extracts were then concentrated further (Gliessman & Miller, 1972). Those laboratory studies which used extracts of bracken material provided variable and often contradictory results on inhibition of plant growth by bracken (reviewed in Den Ouden, 1995). Toxicity of bracken in allelopathy experiments was often dependent on the extraction time used in the preparation of bracken extracts. Den Ouden (1995), regards it entirely as an artefact of prolonged extraction, quoting the words of Harper (1977, p.369): '*almost all species can, by appropriate digestion, extraction and concentration, be persuaded to yield a product that is toxic to one species or another*'.

The extraction problem has also surfaced in animal experiments. Evans *et al.* (1984) conducted an experiment in which water was percolated through bracken in glass aspirators for two weeks and the run-off fluid was administered orally to mice. Hepatoma, gastric squamous papilloma and lymphocyctic leukaemia were induced. This experiment is relevant to this study because the method of leaching the toxins mimicked that of rain. However, the system of circulation was closed, with addition of toluene necessary to prevent fermentation. Rainfall in the uplands would result in a rapid flush of toxins through the stream, augmented by the high flow, an inevitable consequence of rainfall in small upland streams. The model of Evans *et al.* (1984) simulates long-term accumulation of toxins in water, which is unrealistic in small fast-flowing streams, but may be relevant when investigating potential bracken effects on small still water bodies.

A more realistic run-off simulation was performed by Taylor & Thompson (1990), where 250 ml of water was sprayed once over undamaged bracken fronds at a density approximating that of local swards, and the run-off, collected after 2 hours, was administered to plant seedlings. Inhibition of radicle elongation was observed in several Australian plant species. The amount of rainfall experienced by fronds prior to their collection reduced the toxic effects of frond washings significantly, suggesting prior depletion of toxins by rain. Also, the run-off from senescent fronds inhibited radicle elongation in one plant species.

The problem of realistic dosage of bracken is best illustrated when addressing work on bracken toxicity to animals. The list of animal diseases caused by exposure to bracken is impressive, confirming the considerable toxic *potential* of this fern. However, as has already been stated (Chapter 1), the diseases induced by grazing on bracken in natural conditions are found exclusively in *domestic* animals, primarily in situations of stress - on poor pastures where other sources of food are not available or very limited. This already shows that exposure to bracken toxins may be an artefact of environmental manipulation by man. The same is mirrored in most of the experimental work - bracken (or its extracts) is often force-fed to experimental animals (Evans *et al.*, 1984), or administered to herbivores in doses which exceed a reasonable figure of possible bracken intake (Prakash *et al.*, 1996). Many studies use animals such as rats, mice and guinea pigs, which do not normally eat bracken. Bracken extracts have even been administered directly into the stomachs of guinea pigs via surgically inserted tubing (Evans *et al.*, 1982) and incorporated into the food of rats at proportions of 33% of its total dry weight (Evans & Mason, 1965). Although the ecological validity is not the aim of such experiments, it is easy to extend erroneously their findings to the ecosystems outside the laboratory and thus overestimate the threat of bracken to animals in natural conditions. The majority of studies in this area confirm *chemical* rather than ecological toxicity of bracken.

In order to extrapolate the toxicity of bracken compounds to the natural situation it is paramount to investigate the extent of normal exposure of animals to bracken or its products, base consequent experimental work on these realistic levels and use relevant extraction methods.

4.1 Aim

The aim of the experiment was to determine whether run-off from fresh, undamaged bracken fronds results in decreased rate of growth in *Gammarus pulex*. The hypotheses are:

H_0: there is no difference in growth beween *Gammarus* subjected to bracken run-off and *Gammarus* in the control group.

H_A: the growth of *Gammarus* subjected to bracken run-off is lower than that in the control group.

4.2 Method

The experimental stream channel was constructed from a fibreglass tank with the following dimensions: length 190 cm, width 50 cm, depth 24 cm. A sheet of perspex was used to split the tank into two 25cm wide channels, one for the bracken treatment and the other for the control group. See Fig.44 for an annotated diagram of the experimental set-up. The flow of water through the experimental channels was kept constant, and a divider junction separated the waterflow for the two channels. The water was drawn from the open tanks on the roof of the building, where tap water is kept prior to being used in aquaria. The outflow pipes discharged into the drains. The depth of water in the channels was 10 cm. A second water supply to the channels was used to create bracken run-off. Again, water was drawn from the roof tank via plastic piping. A tee-junction divided the waterflow, which for one of the channels went into a shower head and then through a pack of bracken fronds in a funnel. The outflow from the funnel emptied into into one of the channels. In order to compensate for the increased flow in the control channel due to the run-off, the second branch of the tee-junction emptied into the control channel. A valve was set up on the control pipes in order to regulate the flow.

Each of the *Gammarus pulex* used in the experiment was housed in an individual cage, which was a stainless steel cylinder wrapped in 0.25 mm mesh, with the same mesh at the base. The mesh was glued to the cylinder with aquarium silicon sealant.

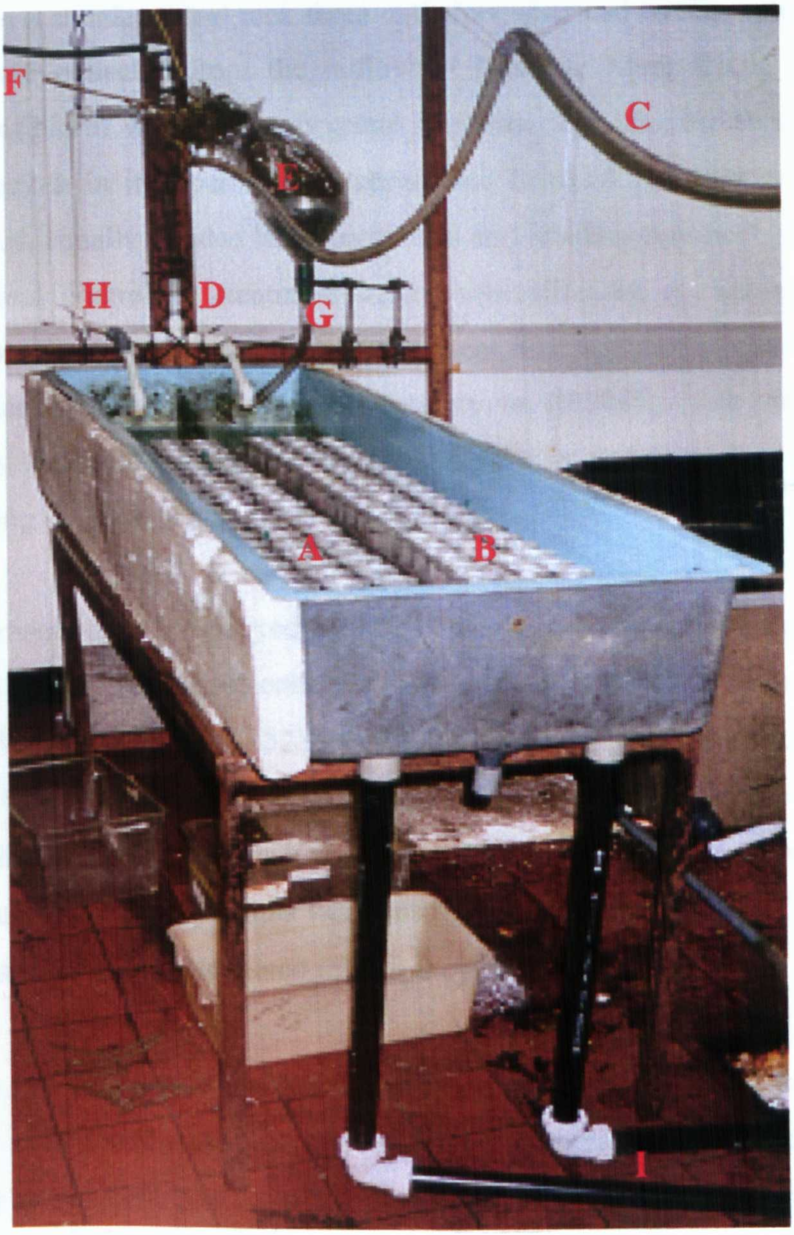


Fig.44 Experimental set-up. A. control channel; B. bracken channel; C. water supply to the channels; D. tee-junction separating the waterflow to the channels; E. bracken fronds pack in a funnel; F. separate water supply to the bracken pack for the run-off; G. outflow from the bracken pack into the channel; H. inflow to the control channel compensating for increase in flow due to bracken run-off I. outflow to the drains.

The cages were 14 cm high, with 5.5 cm diameter base. In the channels the cylinders were housed in a stainless steel rack three cylinders wide and twenty cylinders long. *Gammarus* were collected from the ouflow of Rosterne Mere (NGR SJ342382), which is part of North West Midlands group of meres. The lake has an insignificant amount of bracken in its riparian vegetation. One hundred and twenty *Gammarus pulex* were used, equally divided between control and bracken treatment. The number of the cage and control or treatment status were allocated at random for each individual *Gammarus*. At the start of the experiment each *Gammarus* was weighed on electronic balance (Sartorius Research), accurate to 0.00001g. The animals were weighed every two weeks during the experiment. Prior to weighing each animal was dried on blotting paper for 1 minute.

Bracken treatment was administered for three 24-hour periods each week. Each time fresh bracken fronds were used, collected immediately prior to use from Brotherton Forest Park, Wirral (NGR SJ734825). Only undamaged fronds were used, ensuring that cuts and breaks were not exposed to water. Submerged leaf detritus of oak and beech was collected from the same location as *Gammarus*. The leaves were ground up in a blender and frozen. Fifty grams were defrosted twice every ten days, mixed with 300ml water and 2.5 ml administered into each cylinder with a pipette.

The positions of the cylinders within the racks were changed every three days with the new position determined using the random number generator in the Excel software package. The experiment lasted for two months from September 7, to November 7 2001.The experiment was terminated at the end of bracken growing season. Originally, *Baetis* mayflies from one of the study streams were chosen as study animals, but these require highly oxygenated water, which resulted in the loss of 2/3rds of all the animals within two weeks. The experiment was then terminated and re-started using *Gammarus pulex*. The difference between the growth of *Gammarus* in the bracken-treated and control conditions was tested by comparing the regression equations fitted to growth data using analysis of covariance (ANCOVA). Straight-line regression was fitted because growth of juvenile *Gammarus* is exponential, although the full life span growth confirms to a logistic growth curve (Sutcliffe *et al.*, 1981). ANCOVA was carried out using the SAS software package. Water chemistry data were analyzed by two-way ANOVA, where each sampling occasion was analysed separately using the Minitab statistical package.

Chemistry of the water in the experimental channels was monitored. Oxygen readings (Microprocessor Oximeter Oxi 196) were taken from the inflow (before the flow entered the cylinders), outlow (flowing out of the cylinders) and from one of the first, the last and the middle rows of the cylinders in the rack. Throughout the experiment pH and conductivity were measured, again, comparing the inflow, the outflow and the cylinders in the first, last, and middle rows. Single measures of nitrate-N, ammonia-N, iron, magnesium and sodium were made from the inflow water on December 5 2001. See Chapter 2 for the methods used. Temperature of the inflow was also measured. Samples of water were also tested for ptaquiloside (see Chapter 2 for method). Three samples were taken from bracken run-off water before it entered the channel: the first immediately after the water started to circulate through the bracken pack, the second 2 hours afterwards, and the third 24 hours after the start of the treatment. Also a sample was taken from the channel itself 24 hours into bracken treatment. In addition a sample of water was from the feeder tanks, and another sample from the run-off from crushed bracken fronds.

4.2.1 The choice of test species and stress detection method

Gammarus is considered sensitive to a range of pollutants (Sloof, 1983), and is a good potential indicator of physiological stress induced by chemical substances. For this reason this species has been widely used in ecotoxicological work, and is as good a candidate as any to exhibit physiological effects induced by bracken toxins. It has also been shown that food consumption by *Gammarus* is reduced under stress (McCahon *et al.*, 1989), leading to a decrease in the amount of energy available for production, and hence reduced growth (Maltby *et al.*, 1990(a,b), Maltby, 1994). Growth of *Gammarus*, or rather its scope for growth (SfG), has been used successively in order to establish the effects of a range of pollutants such as zinc (Maltby & Naylor, 1990), iron, manganese (Maltby, 1994) and chlorinated esters, which are petrochemical pollutants (Maltby, 1992). Scope for growth assay calculates, out of the ingested total, the amount of energy allocated to production, accounting for that used for metabolism, and for the loss via excretion.

4.3 Results

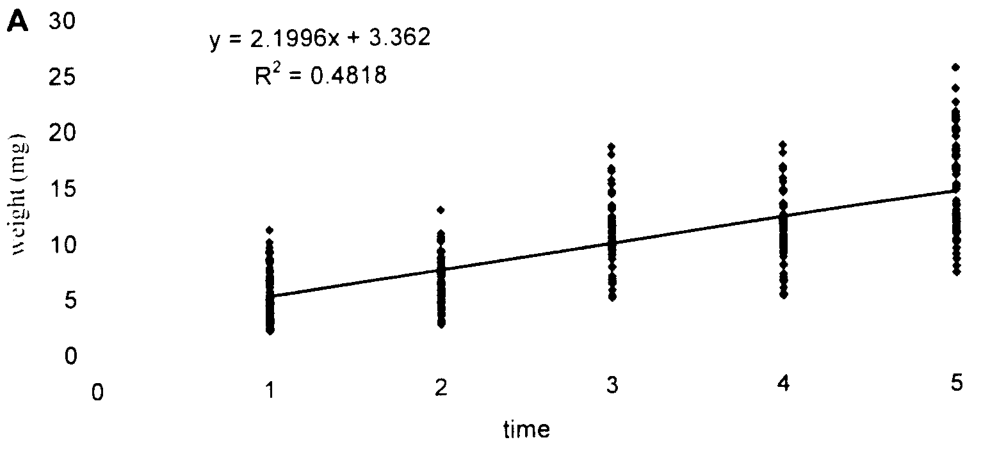
Out of 60 *Gammarus* the growth data for the controls were obtained from 55. Three individuals died, one as a result of mishandling while being weighed. One *Gammarus* escaped from its cylinder, and one wedged itself in the silicon sealant and could not be extracted to be weighed, although it survived for the entire duration of the experiment. In the bracken treatment growth data were obtained from 58 individuals. One of *Gammarus* died, and one escaped. During the course of the experiment the mean weight of *Gammarus* increased more than two-fold, but growth continued. The initial and final weights of *Gammarus* are presented in Table 15.

Table 15. Change in the bodyweight of *Gammarus pulex* during the experiment.

	Mean initial weight (mg) ± SD	Mean final weight (mg) ± SD
Control	6.28 ± 2.75	14.25 ± 4.01
Bracken	5.90 ± 2.33	14.84 ± 4.43

The combined plots of the increase in *Gammarus* bodyweight during the experiment are very similar for both bracken treatment and control group (Fig.45). The equations of the fitted regression lines are also very similar $y = 2.2x + 3.62$ for bracken treatment and $y = 1.92x + 3.76$ for the control group. ANCOVA showed no significant differences between either the slopes ($F = 2.2$, d.f.= 1,109, $p = 0.139$), or the intercepts ($F = 2.08$, d.f.= 1,110, $p = 0.15$) of the two regression equations. The differences in *Gammarus* growth were, therefore, not significantly different, and the null hypothesis is accepted. There was, therefore, no inhibition of growth by bracken run-off.

Temperature of the water in the inflow varied between 13.5 and 15.2C°. On each sampling occasion it was identical in control and bracken channels. The differences in pH and conductivity between control and bracken channels were minimal. pH was slightly alkaline, varying between 7.27 and 7.67 in the control and 7.3 and 7.67 in the bracken channel. Two-way ANOVA showed no significant differences between the cylinders situated in different positions in the rack, but on four (out of 14) sampling occasions pH was significantly higher in the control channel ($p < 0.05$). Conductivity ranges were almost identical for the control and bracken channels, between 86 and 158 µS/cm in the former and 86 and 157 µS/cm in the latter. There were no significant differences on any sampling occasions either between the positions or



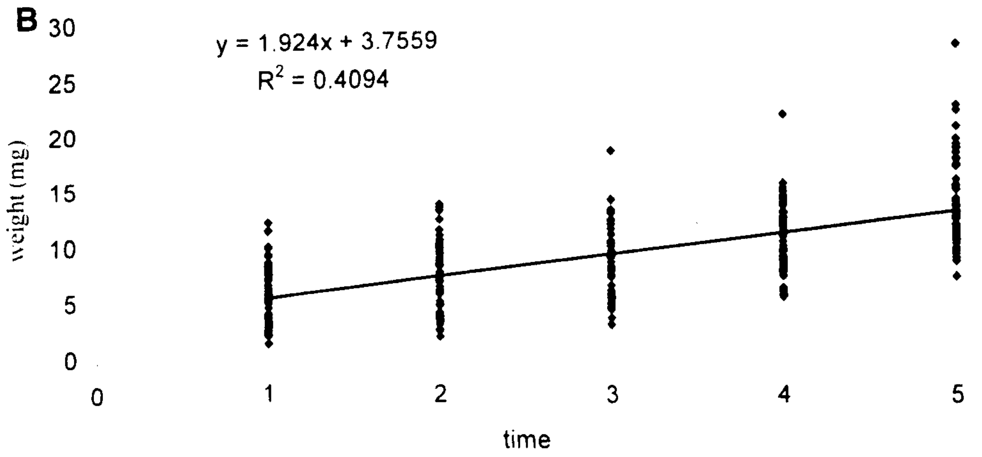


Fig.45 Growth of *Gammarus pulex* subjected to bracken run-off (A) and in the control group (B). The time interval between weighing occasions was two weeks.

between channels. Oxygen concentration decreased down the flow of the channel. Position of cylinder was significant on three out of four occasions when oxygen levels were measured. Differences between channels were not significant. See Table 16 for levels of other chemical determinands measured. No traces of ptaquiloside were detected in any of the water samples from the experimental channels. Ptaquiloside was, however, detected in water run through crushed bracken fronds.

Table 16. Chemistry of the water used in the experiment.
Based on a single measurement.

Determinand	Concentration
NH_4-N (µg/l)	34.22
NO_3-N (mg/l)	0.26
Na^+(mg/l)	4.33
Fe(mg/l)	0.12
Ca^{+2} (mg/l)	8.5
Mg^{+2} (mg/l)	1.25

4.4 Discussion

The results provide no evidence that *Gammarus pulex* was affected by the run-off from fresh fronds. Bracken run-off did not alter the chemistry of the water in the channel, although mean pH was on four occasions greater than in the control channel. These occasions did not, however, coincide with, or follow bracken treatment. General water chemistry was not greatly dissimilar from that in upland streams.

Three scenarios are possible. No release of toxins occurred. Release of toxins did occur, but they had no effect on *Gammarus*. Release occurred, but the deleterious effects were not detected. The first scenario is supported by the fact that ptaquiloside was not detected in the bracken channel and in the run-off from the undamaged fronds. Ideally, a range of toxins should be tested for, but it is an expensive process requiring specialised equipment. Ptaquiloside was, therefore, used as an indicator of the total toxin content of bracken.

Lack of ptaquiloside does not, however, rule out that other toxins were not leached out, and these may or may not have been detected by measuring growth. However, *Gammarus* are known to reduce their feeding under stress, including that induced by toxins (McCahon, 1989). Reduced feeding by *Gammarus* over the period of two months would likely have been translated into a weight loss detectable by the methods used in this study. Duration of the experiment may also have affected the results. *Gammarus* continued to grow when the experiment was terminated, and had the experiment been continued until *Gammarus* reached their full size, the growth curves may have eventually diverged, with the effects of reduced feeding under stress manifesting themselves later. Sutcliffe *et al.*, (1981) reported life span of *Gammarus* as between 350 and 450 days, with some individuals living up to 700 days when kept at 15C° and fed on elm leaves. Maximum bodyweight was between 50 and 70 mg. It is difficult to say accurately how old the animals used in this experiment were, but the average bodyweight at the end of the experiment was less than 15mgs suggesting the approximate age at the end of experiment as four months, using Sutcliffe's *et al.*, (1981) growth curve. Also the growth rates of males and females at this stage do not diverge (Sutcliffe *et al.*, 1981), which is important, as the animals were not sexed prior to experiment. The growth curves within treatments, however, did not show divergence into faster and slower, which could have been attributed to different growth rates between sexes.

There still remains a possibility that cellular and subcellular effects did take place, but their detection is outside the scope of this study and this experiment. As the number of toxins in bracken is so large, so would be the number of potential effects on tissues and organs of invertebrates. As many of the toxins are mutagens, then at least a potential must exist for the ability of these toxins to affect the DNA of invertebrate animals, by forming adducts, as well as that of mammals.

The most likely conclusion is that toxins (at least ptaquilosde) are locked in the bracken tissues and are not removed from undamaged fronds by sprayed water (rain). The results of this experiment have given additional weight to the argument that bracken fronds, without undergoing chemical and physical treatment, such as maceration, are inert and do not adversely chemically affect organisms that are in contact with it, although spore inhalation may be an exception. Presence of ptaquiloside in run-off from crushed fronds raises the question of the possible risk of unlocking bracken toxins by mechanical methods of bracken control, such as cutting or crushing by machinery. Toxins from the exposed tissues may then be leached out into soil and groundwater. The action of rain on intact fronds, however, appears insufficient to leach toxins into streams.

"Ваш роман прочитали, - заговорил Воланд, поворчиваясь к Мастеру, - и сказали только одно, что он, к сожалению, не окончен."

Mikhail Bulgakov
Master and Margarita

CHAPTER 5

Conclusions

5 Summary and conclusions

The modern upland ecosystem in Wales is a product of man's activity which began with the destruction of woodland two millenia ago, followed by maintenance of grassland and moorland which continues to this day. In the past two hundred years acidification from industrial sources and recent afforestation by exotic species completed the formation of modern upland ecosystem. The impact on upland freshwaters has been immense. Shaded, retentive woodland streams supplied with leaf litter and maintaining fish populations were transformed into exposed, detritus limited, largely fishless and highly acidic water bodies. The emergence of bracken as one of the main upland vegetation types may have contributed to the effect of man-made terrestrial vegetation change has had on the streams in the uplands.

The survey approach of detecting the effects of bracken involved two groups of streams of very unequal size - ten Berwyn compared to three Clwydian, which led to pooling of their data. Originally the Clwyd group of streams was supposed to include four other neighbouring catchments, which would have been analysed as a separate dateset. On closer inspection the other streams were strongly influenced by human activity, being surrounded by farmland, and were thus excluded from the survey. The analysis of combined Berwyn and Clwydian streams resulted in artificial non-causative correlations between some variables, but the analysis of the Berwyn data yielded the same results in terms of variables influencing the community composition for both invertebrates and diatoms. In agreement with established opinion, the water chemistry variables, especially those connected with acidity, were the most important factors (Ormerod *et al.*, 1993). The physical variables that emerged as significant were often correlated with chemical variables.

Clwydian streams were shallower and narrower, with greater amount of moss, poorer in periphyton chlorophyll and with lesser habitat complexity, than those in the Berwyns. Clwydian streams had water chemistry characteristics of more lowland waters, with greater alkalinity, higher concentrations of buffering ions and as the result lower acidity. Both Berwyn and Clwydian streams had within - group variation, especially in waterchemistry, determined by local variation in soils and geology. Differences in the amount of bracken present in the catchments are attributable to a combination of soil suitability, altitude and the stage of the on-going bracken invasion and establishment in the catchment.

Bracken biomass was not a factor in influencing invertebrate community composition, and the significance of bracken density was thought to be due to its correlation with chemical characteristics of humic streams. This, combined with the results of the experiment, suggests that there are no toxic effects exerted by bracken on invertebrates and diatoms. Even though the amount of watersoluble toxins in bracken is great, they are likely to be locked in the tissues without leaching into the environment. The results do not agree with those of allelopathy studies where bracken run-off is toxic to a variety of biota (Gliessman & Miller, 1972; Taylor & Thompson, 1990). Damage to the fronds does, however, release the toxins, but such large-scale physical destruction of bracken in the uplands is an unlikely event. The results of the study also give no support to alleged possible contamination of water supplies in North Wales by bracken carcinogens.

This study brings to attention the ecological role of bracken as a food resource in upland streams. The dominant plants in the uplands such as purple moor-grass (*Molinia caerulea*), mat grass (*Nardus stricta*) rushes (*Juncus spp.*) and heather (*Calluna vulgaris*), are fibrous and their detritus is unlikely to be of high nutritional value to shredders (Dobson *et al.*, 1995). Ferns in general, and bracken in particular, are also notoriously poor as a food resource, and avoided by both insect and mammal herbivores (Cooper-Driver, 1977), but how bracken would compare with upland grasses as a food resource for aquatic shredders is not known. Dobson *et al.*, (1995) suggested invertebrate productivity in moorland streams to be limited by the amount of plant detritus. Bracken, may therefore further undermine the already scarce food-base of shredders. On the other hand, if the nutritional value of bracken is higher than that of upland shrubs and grasses, bracken presence in the catchment may improve the quality of allochthonous organic matter in the stream. Where bracken is well-established it will certainly improve the quantity of organic matter. Bird & Kaushik (1987) reported rapid colonisation of fern packs (species not stated) by non-shredding invertebrates, and Dobson & Hildrew (1992) suggested leaf litter as limiting as a habitat resource as well as a source of food. If bracken is a low-quality resource, large quantities of its litter transported into streams as the fronds die off may be important both as a low-quality, but abundant food resource as well as a habitat for invertebrates. High density of *Gammarus pulex* in two Clwyd streams where bracken is the dominant vegetation type in the catchment may be a result of this.

Rather than pursuing the question of bracken toxicity in water, it would be beneficial to focus on feeding preferences of upland shredders and compare the attractiveness of bracken as a food resource to other upland vegetation. Growth of invertebrates fed on bracken should also be compared to those feeding on alternative upland vegetation. The experiment conducted in this study was crude in terms of toxic stress detection. Information on the effects of any of the bracken toxins on invertebrate physiology may have provided better indicator of stress than reduction in growth rate.

The fact that toxic effects on invertebrates were not detected in the survey many be explained by the long-term evolutionary view. The presence of bracken (outside woodland) in the uplands probably dates back to first large-scale woodland clearances. Now, more than two millennia since, the biota most sensitive to bracken toxins may have been eliminated altogether, and those that are present may have been tolerant to bracken stress to start with, or have been selected for resistance to bracken toxins. The current fauna, therefore, may be the product of these processes, similar to the way in which acidification has formed the modern invertebrate and diatom communites in the running waters in the uplands.

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