Barium and heavy metals in the waters and aquatic invertebrates of the catchment of bleaberry gill, North Yorkshire

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BARIUM AND HEAVY METALS IN THE WATERS AND AQUATIC INVERTEBRATES OF THE CATCHMENT OF BLEABERRY GILL, NORTH YORKSHIRE

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ABSTRACT

A preliminary survey of Bleaberry Gill, the main stream draining the mining area west of Langthwaite, Arkengarthdale, N. Yorks., gave information on its aqueous metal concentrations and those of associated tributaries. From this, sites were chosen for a more detailed survey of the water chemistry and fauna. Bleaberry Gill was polluted by barium and lead, and, to a lesser extent, zinc. The fauna was similar to that of other 'unstable' upland streams. Low abundance or absence of some groups may have been due to metal pollution, but probably involved other physical and chemical factors.

Metal concentrations in *Baetis* sp(p). were determined for a wide range of aqueous metal concentrations. Positive linear relationships were found between the barium concentration in *Baetis* sp(p). and 'total' water from all sites, and from Bleaberry Gill alone. No relationship was found between the concentration of zinc and lead in *Baetis* sp(p). and 'total' water. It was concluded that *Baetis* sp(p). are unsuitable for use in monitoring aqueous zinc and lead but may be of some use in monitoring barium.

Gammarus pulex was transplanted from a low barium stream 'Mossy Thorn Gill' (0.33 - 0.43mg l^{-1} Ba) to a laboratory tank with 6mg l^{-1} aqueous barium (with no change in other stream characteristics) and to 'Moor Intake Gill', a stream with high aqueous barium (28mg l^{-1}). *G. pulex* took up barium to a concentration significantly above the initial concentration after 4 hours in 28mg l^{-1} Ba and 36 hours in 6mg l^{-1} Ba. An equilibrium was reached after three days, the concentration being higher in animals exposed to 28mg l^{-1}. The rate of uptake appeared to be related to the aqueous barium concentration. It was concluded that *G. pulex* may be suitable for use in the detection of high aqueous barium pollution, or lower level contamination of more than one day duration.

ABBREVIATIONS

μm	micrometre
mm	millimetre
cm	centimetre
m	metre
km	kilometre
ml	millilitre
l	litre
μg	microgramme
mg	milligramme
g	gramme
wt	weight
M	molar
conc.	concentrated
t	time
^{o}C	degree Celcius
n	number of measurements
$\bar{x}$	mean value
S.D.	standard deviation
CL	confidence limits
CV	coefficient of variation
P	probability
v/v	volume for volume

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CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Mining in North Yorkshire for metals such as zinc, lead and copper ceased at the end of the nineteenth century. The mines and mills were abandoned and many have been left undisturbed for the last one hundred years. Although the mining may have ceased there is still evidence of this past activity in the drainage waters of these areas. It is known that streams draining old mining areas can contain elevated levels of metals, such as zinc and lead, for considerable periods of time. Some of the first work on the effects of such pollution was carried out on the River Ystwyth by Carpenter (1922, 1924) and Jones (1940, 1958). This river, in north Cardiganshire (now Dyfed), remained seriously polluted by zinc for thirty-five years after the cessation of mining activities (Jones, 1958). Zinc levels in the main stream near Aberystwyth were still 0.2 - 0.7mg l^{-1} in 1958, whereas the lead levels were almost negligible, having fallen from a 1921 level of 0.4mg l^{-1} in the lower reaches.

One area mined for lead until the 1880s is situated west of Langthwaite in Arkengarthdale. The landscape is one of largely invegetated spoil tips, of waste gangue material, within an area of upland moorland/grassland (Fig. 2.3). Barite (barium sulphate) forms nearly 50% of this spoil (Section 3.7), associated with witherite (barium carbonate) and fluorite (calcium fluoride). Although these barium minerals are largely insoluble, drainage water passing through the tips has been found to carry elevated levels of barium (Say and Whitton, 1982).

A mining company has plans to rework this spoil for barite. Such an operation is bound to have some impact on the environment; it is most likely to affect the drainage systems of the area. Bleaberry Gill, the main stream draining this area, and the springs draining the Fourth Whim tip complex, which is to be reworked (Fig. 3.1), are subject to fairly rapid increase and decrease in flow. This will affect the impact of any additional release of metals into the drainage system. It is possible that the flora and fauna will be subject to very varying aqueous metal concentrations.

As mentioned above, a survey of the drainage of this area (Say and Whitton, 1982) showed that many small streams, particularly those arising in the region of the Fourth Whim tips (Fig. 3.1), carried elevated levels of barium and lead. The aim of this present work was to look at the situation in more detail, with particular regard to the freshwater fauna.

Surveys of the water chemistry alone in the measurement of water quality have their disadvantages. Water samples show the situation at a particular moment and are therefore of little value in the monitoring of a fluctuating metal discharge. A pulse of high metal concentration may pass rapidly downstream one day and may be missed when a water sample is taken the following day. Animals and plants, however, remain in the stream over a longer time period and therefore experience the changes in aqueous metal concentration due to changes in the discharge from a mining operation, in the time of day and in the seasons. Recent work has concentrated on the effects of elevated metal concentrations on the freshwater biota, with a view to the use of organisms in monitoring metal pollution.

1.2 METALS IN THE AQUATIC ENVIRONMENT

Potentially toxic chemicals such as heavy metals can be released into the aquatic environment by natural processes such as the weathering of rocks and volcanic activity. Many, such as manganese, iron, copper, zinc and molybdenum, are essential for life in small amounts. Lead is not known to be an essential trace element in animal nutrition. Industrial processes have, however, greatly increased the mobilization of many of these metals (Mason, 1981) which, in higher concentrations, are known to be toxic to animals and plants. Water is an excellent medium for the dispersal of toxic chemicals. Deliberate discharge or seepage of such pollutants into the water course can cause problems for some distance downstream of the input. It is therefore important to study the effects of these pollutants on the freshwater biota.

There has been considerable work on the effect of the heavy metals, zinc, lead, copper and cadmium, on freshwater organisms, particularly fish (McKim *et al.*, 1973, 1974, 1975, 1976). Initial studies on the invertebrate fauna concentrated on the species composition of water bodies subject to heavy metal pollution. Carpenter (1922, 1924) and Jones (1940, 1958) looked at the effects of seepage from dumps, left by zinc and lead mines, on the composition of the stream fauna. Later studies have looked in more detail at the accumulation of these metals by organisms and their toxicity. A very useful review of heavy metals in the aquatic environment is Förstner and Wittmann (1979) which includes information on toxicity and bioaccumulation of heavy metals in freshwater and marine environments and also has an extensive bibliography. Burrows (1981) has also provided a good review.

Most studies on the occurrence and ecological impact of metals have been concerned with zinc, lead, copper and cadmium; barium has been largely neglected. This study is therefore one of the first to investigate barium accumulation in freshwater invertebrates.

1.21 Mechanisms of metal uptake and loss

Aquatic invertebrates are exposed to metals from three sources:

(a) water

(b) sediment

(c) food

The uptake mechanism is likely to differ according to the source of the metal and species of animal. There are relatively few detailed investigations into the mechanisms of metal uptake and loss by freshwater invertebrates; marine species have received more attention (Förstner and Wittman, 1979).

Trace metals may be adsorbed onto the body surface, incorporated into the body tissues after ingestion with food, or removed from solution via the gut or gills. These metals may then be subject to varying degrees of regulation by excretion or sequestration.

Water as a source of heavy metals has received most attention, probably due to a better understanding of and ability to manipulate water chemistry. It is also initially the most obvious factor to study, as it is common to all stream invertebrates. Bryan (1976), with evidence from marine examples and a freshwater crayfish (Bryan, 1967), suggested that, in the majority of cases, food and particles were more important sources of metals than water. However, it may be dangerous to generalize from his evidence, as much of it was based on fairly large animals and obtained with the aid of radioisotopes under normal rather than metal contaminated conditions.

Metals may be taken up and lost by passive and/or active processes. Many studies have involved the relationship between metal uptake and metabolic activity. Getsova and Valkova (1962) exposed insect larvae to solutions of radioisotopes (Fe, Zn, Cd) at different temperatures. They recognized that uptake mechanisms may differ between insect species and may be, in part, related to metabolic activity. Carter and Nicholas (1978) demonstrated metabolism to be important in zinc uptake by larvae of *Simulium ornatipes* and, since passive ion exchange was shown to be unimportant in the loss of zinc, they suggested that it must be lost by an active processs, such as excretion. In contrast, Kormondy (1965), working on the uptake and loss of ^{65}Zn by nymphs of the dragonfly *Plathemis lydia*, concluded that metal uptake was by surface adsorption or cation exchange, metabolism being quantitatively unimportant. The rates of loss of ^{65}Zn from live and dead larvae were found to be the same, under both laboratory and field conditions, indicating loss to be a passive process. Clubb *et al.* (1975a) showed indirect evidence for metal uptake by aquatic insects being, in part, an active process. They investigated the effect of different oxygen concentrations on cadmium uptake and found that the concentration of cadmium in larvae related positively to the oxygen concentration at which they were maintained. Wright (1980) studied cadmium uptake in the amphipod *Gammarus pulex*, exposing intermoult specimens to ^{109}Cd in artificial stream water. Metal uptake was reduced by 80% when exposed to a metabolic inhibitor (2:4 dinitrophenol), showing that cadmium uptake was largely an active process. Wright suggested that cadmium accumulation may be 'accidental' uptake associated with the calcium regulatory mechanism in which cadmium substitutes for calcium.

Accumulation is a function of both uptake and elimination (active and/or passive) which occur simultaneously. The level measured will depend on the balance between factors determining the two processes. The ability of certain species to withstand the effects of pollution by toxic metals may be related to their ability to prevent such substances accumulating or to allow storage at specific sites. Also, although the rate of uptake may be related to the external concentration, there is no certainty that the concentration in the organism would reflect that of the environment; some species may be able to excrete a higher proportion of the metal intake under metal contaminated conditions. Thus the situation is complex and many studies need to be carried out with different species and metals before the situation is fully understood.

1.22 Factors affecting metal uptake and loss

Most previous work has been concerned with metal uptake and loss from solution; it is likely that the general principles apply to accumulation from other sources. The following factors are important:

(a) duration of exposure

(b) concentration of metal to which the animals are exposed

(c) temperature and oxygen

(d) other metals

(e) biological factors

(a) Duration of exposure

In general the heavy metal concentration of animals increases with exposure. There are two known patterns of heavy metal uptake. The most common situation is for the metal concentration to increase with increased exposure up to a limit after which there is apparently no increase. Some workers have reported that metal concentrations in experimental animals stop increasing after a given time so that an equilibrium is reached. Kormondy (1965), in his experiments on ^{65}Zn uptake by *Plathemis lydia*, reported an equilibrium after 24-48 hours.

Kinkade and Erdman (1975) showed that cadmium uptake in a simulated freshwater ecosystem tended towards an equilibrium as the experiment proceeded. However, these experiments were in closed systems where dosing solutions were not replenished. It is possible that the equilibria reported could be due to a limit of uptake within the experimental system. Some workers did renew the dosing solution and found that, after an initial rapid increase in the concentration of metal in the organism, the rate of accumulation settled to a near constant rate. Gillespie *et al.* (1977) replenished the dosing solution only once, at 96 hours, and showed the rate of accumulation of cadmium by the crayfish *Orconectes propinquus*, from 100ppb and 1000ppb solutions, to decrease after about 20 hours but then remain constant up to 190 hours when the experiment was terminated. They also found that crayfish could accumulate significant concentrations of cadmium, in a time dependent manner, from water containing acceptable levels.

(b) <u>Concentration of metal to which the invertebrates are exposed</u>

An increase in the aqueous metal concentration usually gives rise to higher concentrations in invertebrates. However, the relationship is not a simple one, depending on many factors, both environmental and biological. Gillespie *et al.* (1977) found that the rate of accumulation of cadmium by crayfish was higher in more concentrated solutions, but a ten-fold increase in the metal concentration of the dosing solution did not give rise to a similar increase in the exposed animals. Nehring (1976) found that the nymphs of the mayfly *Ephemerella grandis* and the stonefly *Pteronarcys californica* accumulated copper, zinc and lead more rapidly as the concentration of the dosing solution increased.

Chemical speciation of metals has received much recent attention. Analysis of solutions containing metals gives the total concentration of that metal regardless of its form. It has become apparent that accumulation varies with different metal species, some being more available than others. Dodge and Theis (1979) exposed larvae of *Chironomus tentans* to solutions of copper of the same concentration but with different chemical species. They showed that copper was concentrated most readily when free cupric ions and a copper-hydroxy complex were the dominant forms of the metal, whereas uptake was inhibited by complexation with glycine and NTA (nitrilotriacetate). Barica *et al.* (1973) found no significant uptake of Fe, Mn, Zn, Cu, Pb or Cr by *C. tentans* in the presence of NTA, EDTA or TPP. More studies are needed in this field on naturally occurring organic ligands.

(c) Temperature and oxygen

The metabolic rate of invertebrates is positively related to the body temperature. It has been mentioned above (Section 1.21) that increasing the temperature and/or oxygen concentration available to the animal has, on occasions, caused an increase in the rate of accumulation. Dean (1974) found that tubificid worms, maintained in ^{65}Zn solutions at 15°C, had higher body concentrations of the metal, after nine days' exposure, than animals kept at 6°C, and lower concentrations than worms kept at 25°C. Similarly, Carter and Nicholas (1978) found that blackfly larvae maintained at 20°C concentrated ^{65}Zn to a higher level than larvae kept at 0°C. However, Kormondy (1965) found that the rate and amount of uptake of ^{65}Zn were independent of temperature (10, 20 and 30°C); these results were consistent with his theory that uptake was largely by surface adsorption. Zauke (1982) discovered that the reduction in cadmium concentration of *Gammarus tigrinus* found in summer was due to temperature. An increase in temperature caused a faster growth rate which in turn resulted in more moults. The animals therefore had less time in which to accumulate the metal between moults. The concentration of cadmium in the animals was therefore more a factor of the water temperature than the aqueous cadmium concentration. Zauke found a different situation in *G. pulex* probably because growth of this species is less intensive in the summer; it takes 3 - 4 times longer than *G. tigrinus* to reach sexual maturity at 20°C.

Temperature can also affect permeability since, at certain temperatures, the cuticle becomes more permeable. This may affect the rate of uptake of metals.

(d) Other metals

It is known that increased water hardness may give protection against the toxicity of heavy metals, and it has become clear more recently that it may also influence metal uptake and loss. Kinkade and Erdman (1975) showed that the hardness component (Ca^{2+} and Mg^{2+}) influenced both the rate of uptake of cadmium and the total cadmium concentration in the test organisms at the end of the experiment. The organisms used in this experiment, in a simulated freshwater ecosystem, were the infusorial snail *Ampullaria poludosa*, the catfish *Corydorus punctatus* and guppies *Lebistes reticulatus*. The authors found that initial cadmium uptake from 0.1mg l^{-1} Cd solution was generally faster in hard water (total Ca^{2+} and Mg^{2+} 150mg l^{-1}) but, after 21 days, cadmium levels were highest in animals from soft water (total Ca^{2+} and Mg^{2+} 0mg l^{-1}). Wright (1980) investigated the effect of calcium concentration on cadmium uptake by *Gammarus pulex*. He found no clear relationship between the rate of whole body cadmium uptake and the external calcium concentration. However, Wright and Frain (1981) showed that an increase in the aqueous calcium concentration resulted in decreased accumulation and toxicity. In a solution of 0.5mg l^{-1} cadmium and 200mg l^{-1} calcium, the experimental animals failed to reach 50% mortality after 120 hours; when the calcium concentration was dropped to 20mg l^{-1} the 96 and 48-hour LC 50 values were 0.12 and 0.68mg l^{-1} Cd respectively. Carter and Nicholas (1978) reported that calcium concentrations of 50 and 100mg l^{-1} did not affect the accumulation of zinc from a 0.1mg l^{-1} solution by blackfly larvae (*Simulium ornatipes*) over a 24-hour period, although an increase in the calcium ion concentration was found to reduce the zinc toxicity (Carter, 1976).

(e) Biological factors

The effect of environmental factors on metal uptake and loss depends on the species considered. Different species vary in their vulnerability to particular pollutants. In a study on fish, Solbé and Cooper (1976) compared the median lethal concentrations, in hard water, of three metals to the stone loach and rainbow trout. They showed the stone loach to be more sensitive to zinc but more tolerant of cadmium; both species showed a similar sensitivity to copper.

The following biological factors could influence metal uptake and loss:

(i) stage of life cycle - size, age

(ii) sex

(iii) tolerance to metals

(iv) membrane permeability

(v) differences in diet

(vi) behavioural responses and habitat selection

(i) stage of life cycle - size, age

Different stages in the life cycle may show different sensitivity to metals. Wier and Walter (1976) found immature snails (*Physa gyrina*) to be three times more sensitive to cadmium than mature snails. Similarly, Clubb *et al.* (1975b) observed insects in their early stages to be more sensitive to cadmium than in later stages. Shuman *et al.* (1977) found that organism size affected uptake of metals by benthic macroinvertebrates. Kormondy (1965) found size did not affect the rate of uptake of ^{65}Zn by *Plathemis lydia* but the amount concentrated was inversely related to body size i.e. smaller nymphs had a larger coefficient of accumulation. Wright and Frain (1981) studied the uptake of cadmium by pre-, post- and intermoult *G. pulex* and showed that recently moulted animals took up cadmium much faster than intermoult animals. An explanation put forward by the authors was that cadmium substitutes for calcium in the exoskeleton. A clear inverse relationship between cadmium uptake rate and whole body calcium was shown by Wright (1980). He also found that animals at moult lost all but 4% of their body calcium; they changed from a negative calcium balance to a state of rapid calcium uptake after the moult.

(ii) sex

Anderson and Brower (1978) found no significant difference in the copper, zinc, lead and cadmium concentrations of male and female crayfish (*Orconectes virilis*) from three natural populations. Similarly, Thorp *et al.* (1979) found no difference in the tissue cadmium concentration of male and female crayfish (*Cambarus latimanus*), exposed to the metal for five months.

(iii) tolerance to metals

Brown (1977, 1978) showed accumulation of copper and lead by *Asellus meridianus* to be influenced by the tolerance of the animal to these metals. It was suggested that metal tolerance may be associated with improved metal storage capability.

(iv) membrane permeability

Beament (1961) found large variation in the rates of evaporation of a range of aquatic insects. Some adult beetles were as waterproof as the more permeable terrestrial insects (e.g. caterpillars), whereas beetle larvae seemed to have no specific mechanism for restricting the passage of water. The cuticles of mayfly nymphs were very permeable to water. The gills of all species examined were much more permeable than the rest of the cuticle. Temperature may also affect permeability (Section 1.22c). Brown (1977) suggested that the concentration of metals in insect larvae from the River Hayle probably reflected the permeability of the animals selected. She found the highest levels in the 'free-living' Trichoptera.

(v) differences in diet

The diet of each species is very important in determining the amount of metal to which it is exposed. It has been thought that metals are accumulated along the food chaim. If this was so, then predator species would accumulate more than prey species. However, research on this trophic level magnification (Enk and Mathis, 1977) so far indicates that heavy metals are not magnified in such a straight-foward manner. Namminga *et al*. (1974), in studying copper, zinc and lead in a pond ecosystem, showed no trophic level magnification. Mathis and Cummings (1973) found bottom sediments to act as a 'sink'; the highest concentrations of some metals were found in the benthos. Leland and McNurney (1974) found this 'sink' effect to be especially important for lead; as sediments have a very high sorptive capacity for this metal. Research on lead has shown concentrations to be generally highest in animals that burrow into or ingest sediment.

Another situation that could occur is for a toxic metal pollutant to act specifically on a particular species. If this group is an important prey species then this may indirectly reduce the survival of other groups of invertebrates. The extent of the effect will depend on the specificity of the food source and the presence of alternative food.

(vi) behavioural responses and habitat selection

It has been mentioned above (v) that sediments can act as a 'sink' to some metals, especially lead (Leland and McNurney, 1974). Thus organisms which choose to live in the sediment may be more exposed to certain metals. Kormondy (1965) concluded that zinc is adsorbed onto the exoskeleton of the dragonfly nymph *Plathemis lydia* in amounts proportional to the degree of organism/sediment contact. The choice of habitat therefore affects the degree of exposure to metals.

Some invertebrates have the ability to detect adverse chemical conditions and behave accordingly. Abel and Green (1981) found that *G. pulex* could avoid and detect zinc in laboratory choice chambers at concentrations down to $1 mg\ l^{-1}$ (the lowest concentration tested).

A pollutant may have a sublethal effect i.e. it impairs the functioning of an organism but does not directly cause rapid death. Lead is known to inhibit enzyme activity, acting at a large number of biochemical sites, and zinc (Skidmore, 1970) reduces the efficiency of oxygen transport across the gill membrane of fishes. The sublethal concentration may affect the behaviour of organisms and reduce their fitness in the population. For example, reduction in oxygen uptake may affect fish swimming performance. Although there are no examples, sublethal metal concentrations may affect invertebrates in a similar way.

Metal accumulation is therefore not a simple situation. Metals can be taken up from several sources and the rate of uptake will depend on many factors. Similarly elimination of metals will depend on the rate of passive loss and the ability of the animals for active excretion or storage.

1.23 Invertebrates as indicators of water quality

The need for biological indicators of water quality arises when the water course experiences intermittent high levels of metals which may not be easily detected by water sampling alone. Plants and animals remain in the stream over a longer time period experiencing all the water chemistry changes. A useful monitor would be one which could integrate the conditions it experiences in a predictable way.

Nehring (1973) thought that to use aquatic insects as biological monitors of heavy metal fish kills, they must:

(a) be more tolerant of the heavy metal than the fish in question

(b) concentrate the toxic metal in relative proportion to the metal content of the water

(c) concentrate the metal pollutant by a predictable factor over a short time period

These three points are important in the general monitoring of water quality.

Before an organism can be used as a monitor it is essential that detailed information is known about the dynamic aspects of metal exchange between the organism and its environment.

Nehring (1976), in studying the two insects, *Ephemerella grandis* and *Pteronarcys californica*, found that they did concentrate the metals zinc, lead, copper and silver in proportion to the occurrence of the metals in the stream. He concluded that, under the conditions of the tests, these aquatic insects appeared to be excellent monitors of heavy metal pollution.

This subject is discussed further in Section 7.31.

1.24 Barium

Barite (barium sulphate) is used in oil well drilling as the primary constituent of muds used to flush drill cuttings out of the bore hole. It facilitates the deposition and recovery of these muds, being particularly suitable since it is insoluble and dense (specific gravity 4.2) (Tagatz and Tobia, 1978). Barite is also used in the manufacture of rubber (Weast, 1975).

Soluble salts of barium are known to be more toxic to mammals than insoluble ones (Spector, 1956). Syed and Hosain (1972) determined LD 50 values in S-W (Swiss-Webster) and ICR (Institute of Cancer Research) mice with three soluble barium salts. They found little difference between the LD 50 values for Ba^{2+} in the form of chloride, nitrate and acetate, although they were progressively higher, indicating $Ba\,Cl_2$ to be most toxic. The two strains of mice responded differently to the barium salts. The LD 50 for the S-W strain was about half that of the ICR strain; 8.49 ± 0.14 and 20.10 ± 0.34mg Ba^{2+} kg^{-1} $\pm$ 2S.D. for barium nitrate.

Lazarev (1959) stated that a single dose of 200mg Ba (in soluble form) would seriously affect man, while 500mg is lethal. Man is apparently one of the more sensitive mammals when the dose is expressed on a per weight basis.

There is recent interest in the possibility that barium in drinking water may have long term harmful effects on human health, particularly that it might be associated with high blood pressure. However, Brenniman *et al.* (1981) compared two communities in Illinois that had drinking with barium concentrations of 7.3 and 0.1mg l^{-1} (all other drinking water constituents were nearly identical) and they showed that there was no significant difference between the blood pressures. There was also no significant difference between males and females of the two communities. The maximum contaminant level allowed for barium in drinking water of the U.S.A. is 1.0mg l^{-1}. The E.E.C. Directive on the quality of surface waters intended for the abstraction of drinking water gives a Mandatory level of 0.1 - 1.0mg l^{-1} (see Hargreaves, 1981).

Very little is known on the effects of barium on aquatic ecosystems. Many studies which have included barium in routine stream and river sediments have made little attempt to analyse the results other than to comment on the probable geochemical origin, e.g. Tsai *et al.* (1978) who sampled the sediments of the Iowa river. Merefield (1976) recorded a peak value of 34000μg g^{-1} in the middle section of the River Teign in Devon. In this case most of the barium was present as barite. Thornton *et al.* (1977), measuring the minus 80 mesh fraction (<210μm), recorded values of 1026 - 3257μg g^{-1} in stream sediments from the Arkengarthdale area. Values for other areas in England were as follows: south of England 0 - 178μg g^{-1}, Wales 144 - 214μg g^{-1}, East Anglia 0 - 178μg g^{-1}. The concentration of barium decreases in areas of younger rocks (since the Jurassic). Haslam (1975) surveyed streams in the Cheviots, measuring the minus 100 mesh fraction, and stated that barium values over 2000µg g^{-1} were believed to be due to mineralization. Values over granites and other volcanic rocks were about 560 - 1300µg g^{-1}, whereas sediments over Silurian rocks were low in barium 30 - 210µg g^{-1}. Say and Whitton (1982) sampled sediments in the study area and found the following:

Bleaberry Gill	0212 - 20	3420	Metals in sediments (µg g^{-1})
,,	0212 - 25	14500	27.01.82
,,	0212 - 49	14000	minus 80 mesh fraction
Wetshaw Bottom	0322 - 99	416	
'Fourth Whim Sike'	0323 - 99	22200	
Moulds Old Level	0328 - 10	2100	

Say and Whitton also took replicate samples from 0212 - 49 and found a marked variation in barium concentration at that one site.

05.11.81	3600	Metals in sediments (µg g^{-1})
15.12.81	2700	from 0212 - 49
27.01.82	14000	
28.02.82	4110	
16.03.82	2070	

Cooke and Morrey (1981), in their study of mine waste soils from 24 sites in the Northern Pennines, gave barium concentrations of 12100 - 16100mg kg^{-1} air dry soil. This can be compared with Swaine (1955) who gave the range for normal soil to be 100 - 3000mg kg^{-1}.

Tagatz and Tobia (1978) studied the effect of barite on the development of estuarine communities and concluded that large quantities of the compound, as discharged in off-shore drilling, could adversely affect colonization by benthic larvae. Their experimental design considered the impact of a barite cover over the substrate as well as a mixture of barite and sediment. The former could result from the settling of drilling muds and the latter from the action of currents and active sedimentation. They showed that polychaete worms were particularly sensitive to barite, with a reduction in abundance and species. The species composition of Gastropod molluscs was not affected but they were less abundant. The barite did not, however, significantly affect the numbers of animals from phyla other than the Annelida and Mollusca. The experiment also showed that a cover of barite affected colonization more than a mixture of barite and sediment.

Very little is known about barium and freshwater systems, especially the biological effects. A laboratory study by Havlik *et al.* (1980), on three species of algae, showed that barium was accumulated on the cellular membrane and other components of the algal cell, which could not be extracted with water or alcohol. This suggests that barium is unlikely to be very toxic to organisms that eat the algae, although there is no experimental proof. Another study showed that some species of desmid (Brook, 1981) actually deposited crystals rich in barium sulphate at each end of the cell; he suggested that this process may have some functional significance.

1.25 Zinc and lead

These two heavy metals have been much studied in recent years. Some comments have already been made on laboratory studies in Sections 1.21, 1.22.

The effect of these metals on species composition was first studied. Carpenter (1924) attributed the absence of Platyhelminths, Molluscs, Trichoptera, Crustacea, Oligochaeta and Hirudinea from streams in Dyfed, Wales to be due to lead mine wastes. Jones (1940) suggested that soluble zinc was probably more important than lead in limiting the fauna of the River Ystwyth, Dyfed. Thirty-five years after the cessation of mining activities Jones (1958) found the species composition to still be reduced. He suggested that the molluscs and Malacostraca were absent because of zinc pollution, whereas the Triclads, Oligochaetes and leeches may have been rare because of the scouring action of mine refuse and rubble.

Absence of molluscs has been recognised by several workers as one of the first stages of pollution by lead mining operations (Carpenter, 1924; Ortmann, 1909; Wurtz, 1962).

Nicholas and Thomas (1978) found that heavy metal wastes from abandoned mines entering the Molongo River, New South Wales, had almost eliminated the fauna for many kilometres downstream. Similarly Brown (1977), working on the River Hayle in Cornwall, found the numbers of taxa at heavy metal polluted sites to be reduced.

Many studies have shown that aqueous concentrations of these metals are often exceeded by those in the invertebrates and sediments; the exact relationship varies with the organism and metal considered. As mentioned previously (Section 1.22e), Namminga *et al.* (1974) found the aqueous concentrations of copper, zinc and lead to be lower than any other components analysed. Concentrations of copper and zinc were higher in the benthos than the sediments, whilst the opposite was true for lead. Eyres and Pugh-Thomas (1978) studied the relationship between sediment and tissue concentrations of lead and zinc in the leech *Erpobdella octoculata* and the crustacean *Asellus aquaticus*. Both species showed a clear trend of increase in tissue lead concentration with an increase in sediment lead concentration ($P < 0.01$). *Asellus* seemed to be able to exclude zinc from its tissues to a certain extent and there was an indication of an inverse relationship between tissue and sediment concentrations, although this was not significant. Brown (1977) measured the concentration of zinc in the water, sediments and invertebrates from the River Hayle. The highest concentrations were found in the 'free-living' caddis larvae, where zinc in the tissue appeared to follow levels in the water. No similar relationship was found in the Odonata and Plecoptera larvae. Solbé (1973, 1977) studied the fish and invertebrates from Willow Brook, Northamptonshire, which was polluted by zinc from a steel works, as well as with sewage effluent. Mayflies, stoneflies and *G. pulex* were absent and zinc concentrations below that recorded in Willow Brook were found to be toxic to *G. pulex* under laboratory conditions. Solbé (1977) showed *Gammarus* to be killed within four days by exposure to 2mg l^{-1} zinc.

The use of aquatic insects as monitors of zinc and lead pollution was investigated by Nehring (1976) (Section 1.23).

1.3 ENVIRONMENTAL BACKGROUND TO THE STUDY AREA

1.31 Climate

The study area falls within the Upland zone of the Northern Pennines which includes all the land east of the Pennine watershed and north of the Aire Gap (Smith, 1976). It is an area of principally rough grazing with a height range of 55 - 636m and an average height of 315m. Table 1.1 gives a general climatic description of the area.

Month	Temperature Air °C	30cm Earth °C	Rain mm	PT mm	% coeff	Sun hrs/day	Day length hrs	Rad mw-hr per cm^2	Ill kilolux hrs
JAN	0.9	2.0	99	-1	-	1.2	9.3	50	55
FEB	1.3	2.0	72	5	-	2.0	11.0	105	115
MAR	3.3	3.2	63	24	-	3.0	13.0	195	235
APR	6.2	5.6	67	44	5.1	4.5	15.3	320	380
MAY	9.2	9.1	70	69	8.1	5.4	17.7	415	495
JUN	12.1	12.6	71	77	8.6	5.5	19.1	450	525
JUL	13.5	14.0	85	76	7.4	4.6	18.3	400	470
AUG	13.4	14.0	108	60	9.9	4.3	16.2	335	400
SEP	11.4	12.0	111	36	5.1	3.5	13.9	240	290
OCT	8.5	9.2	106	15	-	2.5	11.7	135	160
NOV	4.3	5.5	107	0	-	1.5	9.9	65	75
DEC	2.2	3.2	109	-3	-	1.0	8.8	35	40
Total	-	-	1068	402	-	-	-	-	-

Latitude : 54.4°N
Growing season : 189 days Apr 25 - Oct 31
Grazing season : 111 days May 10 - Aug 29
Degree-days above 10°C May to Oct : 445
Winter degree-days below 0°C : 260
Grass drought factor : Under 5 days
Mean last frost : Late May

Table 1.1 Areal averages for the Upland Pennine zone 1941 - 1970 (reproduced from Smith, 1976, Agricultural Climate of England and Wales)

The total average rainfall in Table 1.1 is similar to Arkengarthdale, although there are some differences in the monthly rainfall totals (Section 3.4).

1.32 Geology

The study area west of Langthwaite, Arkengarthdale (Fig. 3.1) overlies Lower Carboniferous rocks of the Yoredale facies and Upper Carboniferous Millstone Grit (Ramsbottom *et al.*, 1974). Where the streams pass through old mine workings the substrate includes mineralized rock and associated gangue material, mainly barite. The principal veins carrying lead ore are found in faults in the Yoredale facies. Zinc, lead and copper ores occur with associated fluorine and barium gangues. This mineralization is epigenetic and hydrothermal in origin, principally in fractures and to a lesser extent in flats along bedding structures. The Yoredale facies (Viséan) is made up of sedimentary cyclothems of limestone, shale, sandstone and possibly coal, repeated in succession. The base of this series is the top of the Great Scar limestone which is so obvious in west Yorkshire e.g. Malham. The Millstone Grit series (Namurian) overlies the Yoredale rocks. Bleaberry Gill flows over Millstone Grit in its upper reaches and then over areas of limestones, shales and sandstones in its lower reaches.

1.33 Mining

Arkengarthdale is one of many mining areas in the Yorkshire Dales. It, along with Swaledale and Wensleydale, formed the North Yorkshire Orefield (Small, 1977) which connected with the Alston Orefield to the north and the Craven Orefield to the south. The North Yorkshire Orefield was mined mainly for lead ore, with some copper and zinc and, more recently, barite, witherite and fluorite.

There is evidence of small scale lead mining in the late Iron Age and Romano-British periods, but no evidence for mining in the Bronze Age (Clough, 1980). Nothing is known about mining in Saxon times and very little in detail in medieval times. The first records relating to mining in the district occur about the beginning of the 13th century; there are extant records for Arkengarthdale dated 1182 and 1285 (Clough, 1980), About 1180 large quantities of lead were exported to Waltham Abbey, Windsor Castle and Clairvaulx Abbey (France) (Shayler *et al.*, 1979). After the Dissolution (about 1540) mines were leased to private proprietors and considerable expansion took place, especially in the latter part of the 18th century when there was an influx of workers from other areas. The Arkengarthdale and C.B. (Charles Bathhurst) setts expanded during this time. Together they covered 25,000 acres, including the study area. Fortunes declined in the 19th century. Low prices obtained for lead around 1830 caused large scale emigration to the cotton mills of Lancashire and the collieries of Durham (Shayler *et al.*, 1979). There was a slight improvement in the 1840s and 1850s until the renewed depression of the 1880s. After 1885, only a few individual mines were left at work and by 1890 production was negligible. Mining in Arkengarthdale ceased in the 1880s.

The methods used to extract the ore from the vein depended upon the topography in the area of mineralization. At certain places in the steep sided valleys the veinstone came almost to the surface and an extraction method known as hushing was used. This method, practised in the study area until the late 18th century, used an artificial dam of turf. The dam was broken and the water allowed to rush down the hillside to scour out the spoil and loose rocks and expose the veinstone. This was a common method in the Arkengarthdale setts, and large scars, or hushes, can still be seen on the hillsides. Shallow shafts were used on the flatter hilltops, and, as the near-surface and readily available material was worked out, the larger companies began to use deeper shafts. These shafts were sunk along the veins and were connected by levels. Gangue material, barite, witherite and fluorite, associated with the ore, was separated from it and left in large spoil heaps. Much of this is still unvegetated (Fig. 2.3).

The production of barium minerals, barite and witherite, began before 1850 (Almond, 1981). They are relatively easy to separate from their associated gangue material since their specific gravities are high (near 4.3). However, sorting from iron minerals can cause problems and it is impossible to distinguish from zinc blende (zinc sulphide) by gravity methods. Almond considered pollution by barite and coarse witherite not to be serious, but there is evidence that powered witherite is toxic. Several ponies died after eating food carried in tubs which had been previously used to carry the mineral at Burnhope in 1928 (Ashburn, 1963). Ingested barium carbonate can react in the stomach of mammals with hydrochloric acid to produce toxic barium chloride.

1.4 AIMS

It is clear that, although the effects of zinc and lead on stream fauna have been much studied, there is a need for work on barium in freshwater systems. Say and Whitton (1982) showed that the drainage of the mining area west of Langthwaite, Arkengarthdale, N. Yorks. carried elevated barium and lead concentrations; it is possible that the proposed mining activity (Section 3.7) may increase these levels. It was considered worthwhile to study the accumulation of metals, particularly barium, by the freshwater fauna of this area.

The aims of this project were to:

(a) obtain information on the general nature of the water chemistry and freshwater invertebrate fauna of the area with a view to choosing sites and invertebrate groups for further study

(b) determine zinc, barium and lead concentrations in a group of freshwater invertebrates taken from a wide range of aqueous metal concentrations, and to compare these with the aqueous metal concentrations at the time of sampling

(c) study the accumulation of barium by one group of freshwater invertebrates.

During the same period a similar study on the flora of Bleaberry Gill was carried out by Owen (1983). Sections 3.7, 4.1 are based on the same information.

CHAPTER 2

MATERIALS AND METHODS

2.1 APPARATUS AND CHEMICALS

All glassware was made of Pyrex glass, except snap-top vials, which were made of boro-silicate glass. Polypropylene bottles were used for collection and storage of water samples for anion analysis. All chemicals used were of 'Analar' grade, except 'Trace metal analysis' grade nitric acid used for acid digestion and acidification of water samples

2.2 ROUTINE LABORATORY PROCEDURES

2.21 Acid washing

All apparatus was acid washed unless otherwise stated. Apparatus was soaked in 4% v/v nitric acid for a minimum of 30 minutes. It was then rinsed in distilled water six times, in deionized water three times and air dried.

2.22 Acid digestion

In order to determine the metal composition of solid materials the metals must be brought into solution. To do this, a known weight of sample was boiled with 5ml of 2M nitric acid for 1 hour. The solution formed was then cooled to room temperature and made up to the required volume with deionized water. Digestion of samples was carried out in 18mm diameter test tubes suitable for use with a Tecam DB-3H heating block.

A preliminary investigation was carried out into the most suitable acid for use in the acid digestion of animal material; it was shown that this was 2M nitric acid (Section 2.5).

2.23 Atomic absorption spectrophotometry

The concentrations of Ca, Fe, Zn, Ba and Pb in acid digest solutions and water samples were determined by flame atomic absorption using a Perkin-Elmer 403 atomic absorption spectrophotometer. Concentrations of Pb of less than 0.1mg l^{-1} were determined by flameless atomic absorption spectrophotometry using a Perkin-Elmer heated graphite atomizer (HGA - 74). Water samples and acid digest samples taken between 30.07.82 and 07.08.82 were analysed using a Perkin-Elmer 5000 atomic absorption spectrometer (except for Ba).

The concentrations of metals in acid digests were corrected against blanks made up at the same time. Acid-matched standards were used in Ca measurements only.

Water samples were shaken before analysis but acid digest solutions were not.

2.3 WATER

2.31 Collection and storage

'Total' water samples were taken directly from the stream, in the main flow, at 1cm below the surface. The snap-top vial to be used was rinsed once with stream water before the sample was taken. This method differed from the standard method used at Durham University (Algology section) in that the water was not allowed to stand for five minutes before the sample was taken. However, there was very little suspended particle material in the water and it would therefore be unlikely that the results were significantly different. Water for 'filtrable' samples was collected from the stream in a 2 litre beaker and allowed to stand for five minutes. The water was filtered through a 0.2μm Nuclepore membrane filter held in a Swinnex filter holder. Before the sample was taken, the syringe used was rinsed three times in stream water, the first few mls through the filter were discarded and the vial was rinsed once in the filtered stream water. Filters were stored in deionized water and transferred to the holder using stainless steel forceps; they were not acid washed.

Water for anion analysis was filtered through a sintered glass funnel (Sinta porosity 2) into a polypropylene bottle.

All water samples were placed in an ice box for transportation to the laboratory, where they were treated as follows: 'total' and 'filtrable' samples were acidified with 2 drops of trace metals analysis grade concentrated nitric acid, from a '50 dropper' pipette, to reduce the pH to below 1.0, and then stored in the dark at 4°C. Water samples for anion analysis were stored in a deepfreeze at -20°C until required.

2.32 Measurement of variables

2.321 Field

pH and conductivity were measured in the field. An Orion Research Ionalyser (407A) with an Orion combination probe was used to measure pH. Conductivity was measured with an EIL MC-1 meter. A subjective estimate of stream flow was made on a 1 to 5 scale.

2.322 Laboratory

'Total' and 'filtrable' water samples were analysed, by atomic absorption spectrophotometry, for five metals (Ca, Fe, Zn, Ba, Pb) in the preliminary and detailed survey of Bleaberry Gill and for four metals (not Fe) in the other investigations.

Sulphate analysis was carried out following the turbidimetric method described in Standard Methods for Examination of Water and Waste Water (American Public Health Association, 1980). Solutions were analysed using a Shimadzu double beam spectrophotometer UV-150-02.

2.4 ANIMALS

2.41 Collection, storage and identification

Invertebrate samples were collected by a standard kick-sampling method (Macan, 1958; Hynes, 1961), using a 1mm mesh net. Samples were then washed through a stack of two sieves (5.6, 1.0mm mesh) with stream water (Burrows, 1981). This removed fine organic and inorganic matter and separated smaller animals from the coarse material remaining in the upper sieve. The contents of the sieves were then put into an enamel tray with a small amount of stream water. Stones and coarse plant material were washed and discarded. In the animal survey of Bleaberry Gill a five minute kick sample was taken from each reach. In this case care was taken to sample throughout the reach and in as many microhabitats as possible. The sampling time for collection of animals for acid digestion depended on the abundance of the animals within the reach.

In the survey of animals from Bleaberry Gill the samples from each reach were not sorted in the field but taken to the laboratory in polythene bags containing sufficient water and air to keep the animals alive. These samples were placed in an ice box for transportation to the laboratory where they could be stored live at 4°C for up to two days.

The invertebrates were identified according to the keys listed in Table 2.1. They were sorted live into the main groups and then preserved for later identification. The preservative was as follows: 500ml 70% alcohol, 10ml formalin, 5ml glycerol. A Watson Barnet dissecting microscope was used. Field identification was limited to the use of those macroscopic morphological features that could be seen with a x 10 hand lens. Thus, although it is possible to identify different *Baetis* spp. in the laboratory, they could not be separated in the field and were collectively referred to as *Baetis* sp(p).

Animals used for acid digestion were sorted in the field. Individuals used for acid digestion were complete, live and in an intermoult stage. They were transferred with stainless steel forceps to petri dishes containing stream water, rinsed in deionized water, dried on filter paper and placed in a labelled vial. These samples were then taken to the laboratory where they were killed with boiling water and oven dried at 105°C for a minimum of 60 hours.

group	author	
Malacostraca	Gledhill, Sutcliffe and Williams	1976
Ephemeroptera	Macan	1961
Plecoptera	Hynes	1967
Hemiptera	Macan	1965
Megaloptera	Kimmins	1962
Trichoptera	Edington and Hildrew	1981
Diptera	Cranston	1982
	Davies	1968
general	Macan	1959

Table 2.1 Keys used for the identification of invertebrates

2.42 Acid digestion and analysis

Invertebrate samples were dried (Section 2.41), cooled in a desiccator and weighed to the nearest 0.1mg. Each sample was then transferred to an 18mm diameter test tube for digestion, which was carried out as previously described (Section 2.22). After digestion the solution was poured into a 25ml volumetric flask. The test tube was rinsed six times in deionized water, the washings being added to the flask. The solution was made up to 25ml, any residue allowed to settle, and then poured into the vial in which the animals had been collected. It was important to use the same vial, as body fluids may have dried on to the glass. These fluids would be of negligible weight but might contain substantial levels of metals. Samples were stored at room temperature until analysis. The concentrations of Ca, Zn, Ba and Pb were determined as previously described (Section 2.23). These values were then converted to $\mu g\ g^{-1}$ dry wt of tissue.

2.5 DETERMINATION OF THE MOST SUITABLE ACID CONCENTRATION FOR ANIMAL DIGESTS

From preliminary work (Section 4.23) it was decided that two groups of animals would be studied in detail, a mayfly group *Baetis* sp(p). and the freshwater shrimp *Gammarus pulex*. A study was carried out to show which concentration of nitric acid would be most suitable for use in the digestion of these animals.

2.51 Method

Large samples (about 400 individuals) of these animals were collected, killed and dried as described above (Section 2.41). They were then ground to produce an homogeneous sample. Sub-samples were taken; about 40mg for *G. pulex* and 20mg for *Baetis* sp(p). Each sub-sample was accurately weighed.

Samples were then digested in the following acid concentrations.

G. pulex	Concentrated, 10M, 5M, 2M, 0.5M, 0.1M
Baetis sp(p).	Concentrated, 5M, 2M, 0.5M, 0.1M

Five replicates were taken for each acid concentration. Digestion was carried out as previously described (Sections 2.22, 2.42) using the appropriate acid concentration. Solutions were analysed for Ca, Zn, Ba and Pb (Section 2.23). An acid-matched standard is one made up with the same concentration of acid as the solution to be analysed.

2.52 Results

The results are summarized in Table 2.2 and represented graphically in Figs. 2.1a-c. *t*-tests were carried out between the results from different acid concentrations.

Gammarus pulex

Nitric acid concentration	n	Ca $\bar{x}$	Ca ±95%CL	Ca CV(%)	Zn $\bar{x}$	Zn ±95%CL	Zn CV(%)	Ba $\bar{x}$	Ba ±95%CL	Ba CV(%)	Pb $\bar{x}$	Pb ±95%CL	Pb CV(%)
conc.	4	103000	520	0.3	501	80	10.1	4300	179	2.6	1600	279	11.0
10M	4	102000	3600	2.2	445	17	2.4	5050	112	1.4	1470	192	8.2
5M	4	110000	3350	1.9	456	30	4.1	4320	237	3.4	1460	124	5.3
2M	4	132000	3920	1.9	465	15	2.0	4120	364	5.6	1380	138	6.3
0.5M	3	149000	8870	2.4	446	10	0.9	3850	439	4.6	1470	297	8.1
0.1M	3	116000	18800	6.5	433	42	4.0	1240	165	5.4	1470	770	21.1

Baetis sp(p).

Nitric acid concentration	n	Ca $\bar{x}$	Ca ±95%CL	Ca CV(%)	ZN $\bar{x}$	ZN ±95%CL	ZN CV(%)	Ba $\bar{x}$	Ba ±95%CL	Ba CV(%)	Pb $\bar{x}$	Pb ±95%CL	Pb CV(%)
conc.	4	6550	1010	9.7	3680	201	3.4	6340	295	2.9	2230	387	10.9
5M	4	5990	544	5.7	3490	371	6.7	7000	421	3.8	2370	313	8.3
2M	4	7310	1300	11.2	3490	153	2.8	7220	89	0.8	2270	62	1.7
0.5M	4	6140	119	1.2	3650	222	3.8	6720	656	6.1	2300	426	11.6
0.1M	4	5840	555	3.8	3610	446	7.8	3250	659	12.8	2170	418	12.1

Table 2.2 Metal concentrations in *G. pulex* and *Baetis* sp(p). digested in a range of nitric acid concentrations ($\mu g\ g^{-1}$ dry wt)

Sample size (n), mean ± 95% confidence limits of the mean ($\bar{x}$ ± 95%CL) and coefficient of variation (CV) are given

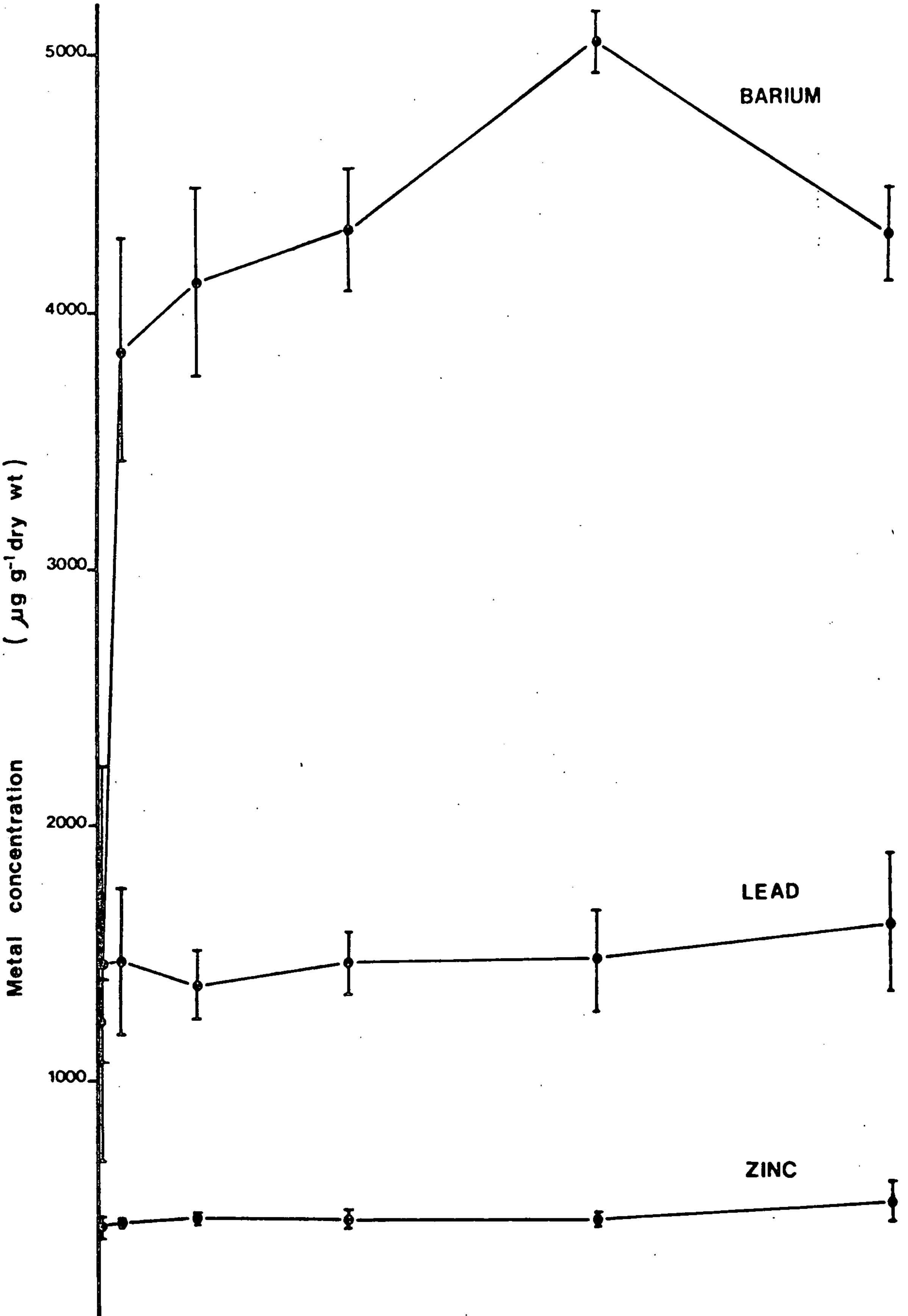


Figure 2.1a Zinc, barium and lead in *Gammarus pulex* digested in a range of nitric acid concentrations. Vertical bars represent 95% confidence limits of the mean

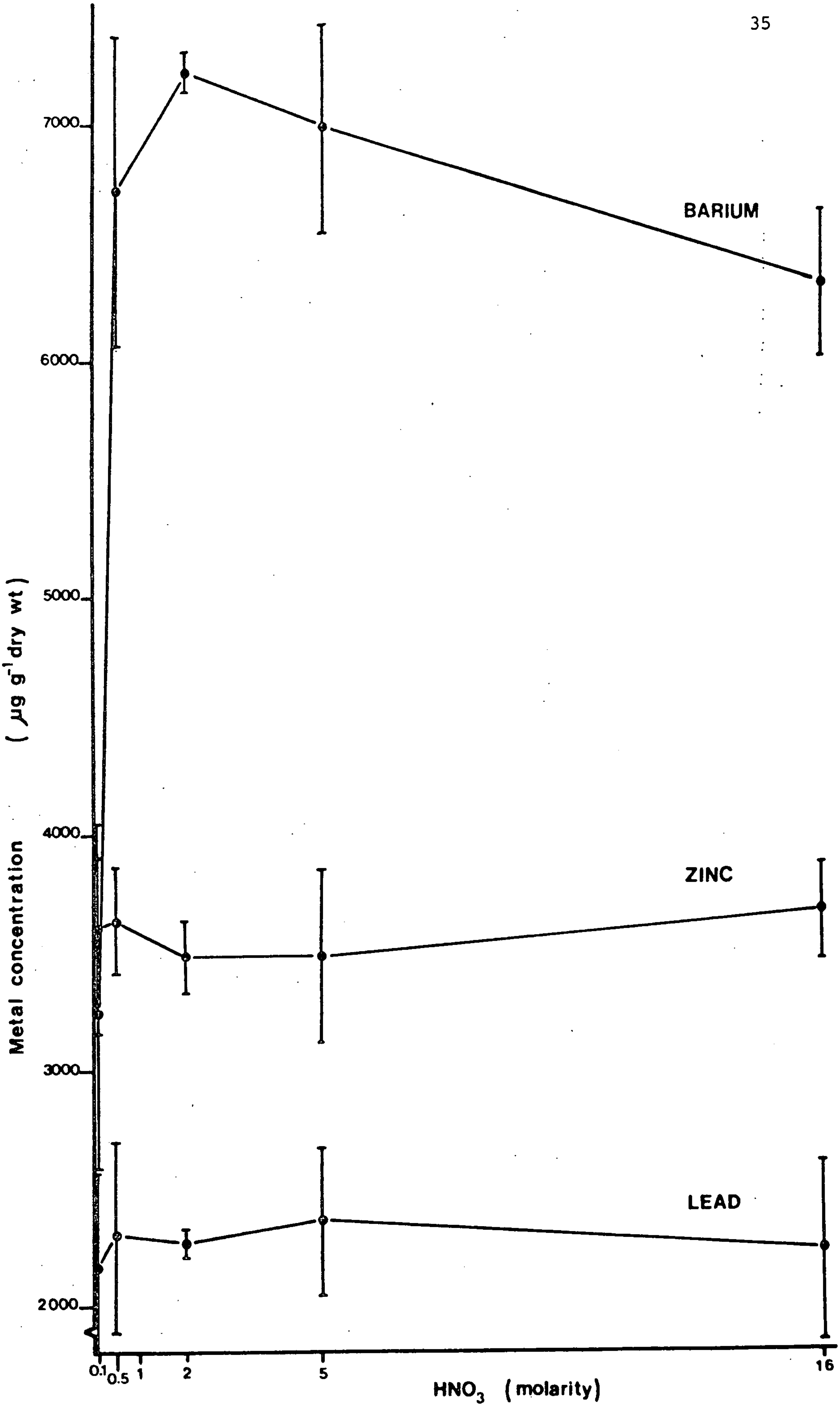


Figure 2.1b Zinc, barium and lead in *Baetis* sp(p). digested in a range of nitric acid concentrations. Vertical bars represent 95% confidence limits of the mean

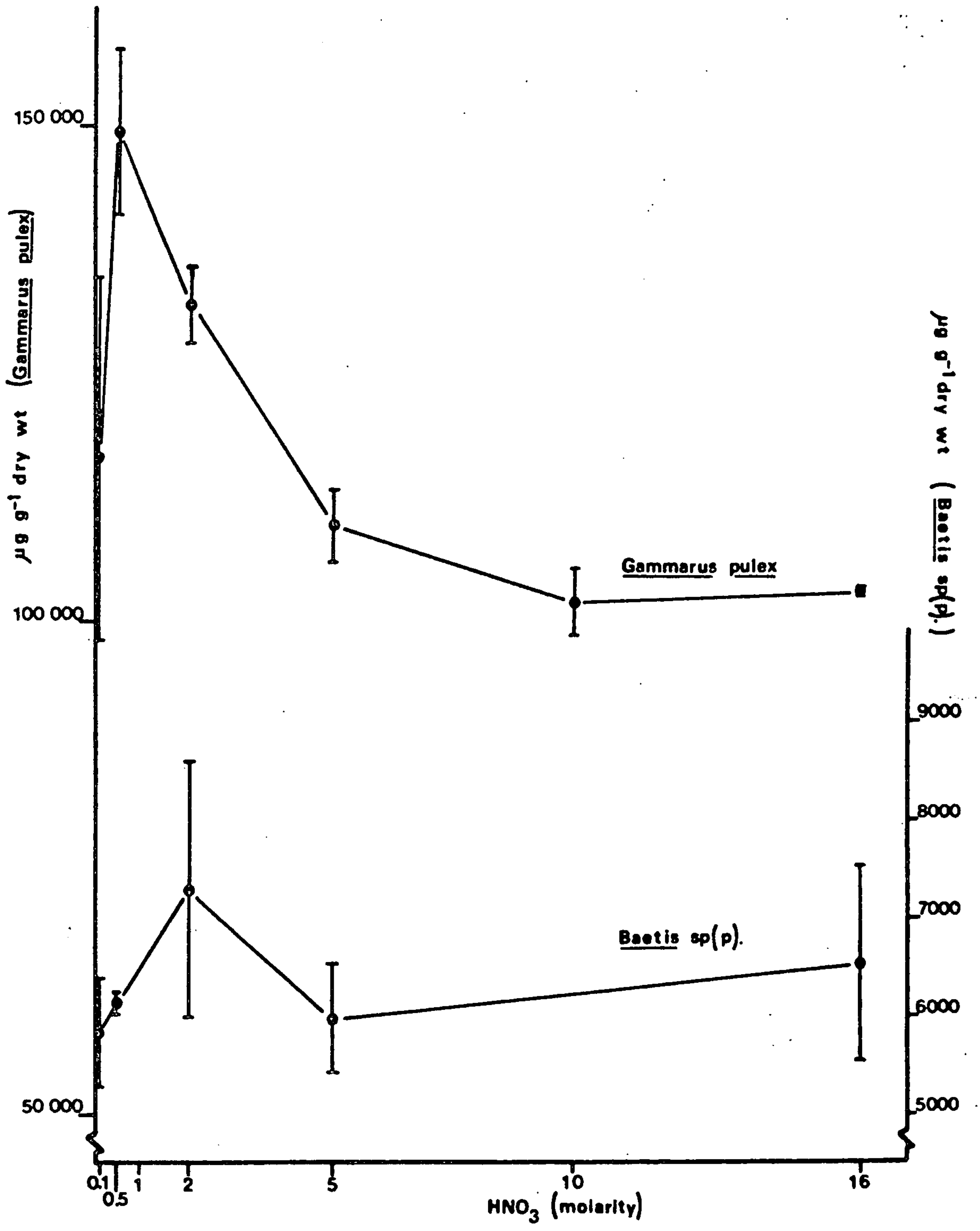


Figure 2.1c Calcium in *Gammarus pulex* and *Baetis* sp(p). digested in a range of nitric acid concentrations. Vertical bars represent 95% confidence limits of the mean

Comments

i) Zinc and lead showed no significant difference between any of the concentrations obtained with either *G. pulex* or *Baetis* sp(p).

ii) The mean barium concentration showed a peak at 10M with *G. pulex*. This value was significantly different from that obtained using 5M ($P < 0.01$) and concentrated acid ($P < 0.01$). There was no significant difference between the barium concentrations obtained using 2M, 5M and concentrated nitric acid.

iii) The mean barium concentration showed a peak at 2M with *Baetis* sp(p). This was not significantly different from the values obtained using 0.5M ($P > 0.10$) and 5M ($P > 0.10$) acid, but it was significantly different from the concentration obtained with concentrated acid ($P < 0.001$).

iv) The highest mean calcium concentration was obtained with 0.5M acid with *G. pulex* and 2M acid with *Baetis* sp(p).

v) With *G. pulex*, 0.5M acid gave a significantly higher calcium concentration than 2M ($P < 0.001$) and 0.1M ($P < 0.01$). With *Baetis* sp(p). 2M nitric acid gave a significantly higher calcium concentration than 0.5M ($P < 0.05$) and 5M ($P < 0.05$).

2.53 Discussion

It was assumed that the higher the measured metal concentration, the more efficient and accurate the method of acid digestion. As can be seen by the results, the 'efficiency' of acid digestion by each nitric acid concentration varied with the species of animal and metal studied. Ideally therefore, the concentration of acid chosen for digestion should depend on these two factors. For practical reasons however, it was necessary to choose one nitric acid concentration.

Any of the nitric acid concenctrations used in this investigation would have been suitable for the release of zinc and lead from *G. pulex* and *Baetis* sp(p). This suggests that these two metals are loosely bound chemically and are therefore released easily into solution.

10M acid gave a higher barium concentration for *G. pulex*, while 0.5 - 5M was most suitable for the extraction of barium from *Baetis* sp(p). Calcium extraction required a lower acid concentration, 0.5M with *G. pulex* and 2M with *Baetis* sp(p).

After two weeks storage, the digest solutions made up with 0.1 and 0.5M nitric acid showed signs of bacterial action. Both sets of solution produced an odour common to that produced by decomposing material, and the 0.1M digest solutions were unusually cloudy in appearance. To be suitable for use in this method the acid used should allow the solutions to be stored for an indefinite period without risk of decomposition. For this reason the two lower acid concentrations were considered unsuitable.

Economy and safety were two other important considerations. Both concentrated and 10M nitric acid are expensive and somewhat dangerous during digestion and analysis.

A balance was therefore needed between all these factors and it was decided to use 2M acid in the digestion of the two groups of animals studied.

Both barium and calcium showed peak mean metal concentrations. It is possible that the acid concentrations below that which produced the peak value did not break down the animal material sufficiently to release all the metals. However, above this peak it could be expected that the metal concentration would remain constant, i.e. all the metal would be brought into solution and made available for detection. The barium concentrations in *G. pulex* and *Baetis* sp(p). and the calcium concentrations in *G. pulex* showed a drop in the level of metal detected as the acid concentration increased. It is possible that there was some detection interference caused by the presence of the high acid concentration. This could be direct interference by the acid or indirect interference caused by the acid changing the chemical nature of other materials in the solution with the subsequent effect of reducing the level of metal detected. The former (direct interference) was corrected for by the use of acid-matched standards in the analysis.

2.6 INFLUENCE OF THE PRESENCE OF GUT CONTENTS ON MEASUREMENTS OF METAL CONCENTRATIONS IN *GAMMARUS PULEX*

About 90 animals were collected from 'Mossy Thorn Gill' (0350) and five replicate samples of eight animals were taken (Section 2.41). A polythene bottle was filled half full with 'filtrable' stream water (using a sintered glass funnel (Sinta porosity 0) to remove large particles). The remaining animals were washed once in 'filtrable' stream water and placed in this bottle. The bottle was then submerged on its side (to allow the maximum air/water interface) in 'Mossy Thorn Gill' for more than 18 hours; this allowed the animals to clear their guts. Five replicate samples of eight animals were then taken from this bottle.

Samples were acid digested as described above (Sections 2.22, 2.42) and were analysed for Ca, Zn, Ba and Pb (Section 2.23).

2.7 BARIUM UPTAKE AND LOSS BY *GAMMARUS PULEX* - laboratory study

Freshwater shrimps were transferred from a stream with relatively low aqueous barium to a laboratory tank with high aqueous barium. After three days exposure the remaining animals were transferred to a laboratory tank with low aqueous barium, for 11 days.

2.71 Barium solution and water used

A solution of about 6mg l^{-1} barium was made up using barium nitrate and 'filtrable' stream water (see below - this section). 6mg l^{-1} was chosen as it was the approximate concentration of 'Moor Intake Gill' (0336-50), on 28.06.82, to which a field transplant was to be made (Section 2.8, 6.3).

Stream water was collected at the same time as the animals. It was transported to the laboratory and stored at 8°C. This water was then filtered with a sintered glass funnel (Sinta porosity 0) to remove large particles, without otherwise affecting the nature of the water. All water used in this experiment was 'filtrable' stream water.

2.72 Collection and storage of *G. pulex*

About 800 animals were collected (Section 2.41) from 'Mossy Thorn Gill' (0350) and transported to the laboratory in a bucket containing water and sediments from the stream. The sample was kept cool by placing the bucket in an ice box. The complete sample (animals, water and sediments) was then transferred to a 'stock' tank and stored at 8°C. This temperature was that of the stream and therefore the animals experienced little change. The photoperiod of the cold room was 18 hours light and 6 hours dark, which corresponded to outside conditions. The stock tank was aerated.

2.73 Experimental design

About 700 animals were transferred from the stock tank to an aerated 'supply' tank. They were left to purge themselves for 15 hours. This was considered necessary since the presence of gut contents could affect the level of barium measured in the animals (Section 6.1). Two tanks were set up: A, no added barium (- Ba); B, with added barium (+ Ba). Five samples were taken, at the start of the experiment, from the animals in the supply tank as described below (Section 2.731). 100 animals were transferred to A and about 600 to B. Both tanks were aerated during the experiment. All dead or dying animals and cast skins were removed from the tanks on each sampling occasion.

2.731 Sampling programme

Samples of animals and water were taken at intervals throughout the three days.

sampling time (t)	A	t + 0, 24, 48, 72 hours
	B	t + 0, 1, 2, 4, 8, 12, 24, 36, 48, 72 hours

A water sample was taken at the same time as each animal sample. Five replicate samples of eight animals were taken at each sampling time. Animals, which had obviously just moulted (and were very pale in colour), were not included in the samples. Deaths were noted throughout the experiment.

2.74 Loss of Barium

After three days exposure to 6mg l^{-1} barium, the surviving animals from B (about 200) were washed twice in water and transferred to A (after first removing the remaining animals in A). One sample of eight animals was taken at each sampling time over the next 11 days.

sampling time (t) +1, 2, 5, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 264 hrs

Water samples were taken at 24-hour intervals.

2.75 Analysis

Animal samples were digested in 2M nitric acid as described in Sections 2.22 and 2.42. The resulting solutions and water samples were analysed for barium by atomic absorption spectrophotometry as described in Section 2.23.

2.8 METAL UPTAKE BY *GAMMARUS PULEX* - field transplant study

Freshwater shrimps were transferred from a stream with relatively low aqueous barium to a stream with high barium, for a period of five days.

2.81 Transplant cage

A transplant cage was designed which would allow a through flow of water but not allow escape of the animals within it or the entry of free animals from the water outside. It took the form of a square plastic bowl with the centres of two opposite sides removed and covered with 1mm mesh nylon netting (Fig. 2.2a,b). A removable nylon mesh cover was designed which prevented material from falling into the bowl.

2.82 Method

About 800 animals were collected from 'Mossy Thorn Gill' (0350) and transferred to the transplant cage (Section 2.81). This was placed in another bowl containing water which had been filtered through a sintered glass funnel (Sinta porosity 0) (Section 2.71) and the whole apparatus was put back into the stream, thus keeping the animals at stream temperature. They were left to purge themselves for >18 hours before the transfer. The cage and contents were then transferred to 'Moor Intake Gill' (0336-40). Washed and scrubbed stones were placed in the cage to weigh it down and to provide some cover for the animals.

2.83 Sampling programme

Five replicate samples of eight animals were taken at each sampling time. Animals which had just moulted, they were very pale in colour, were not included. Sampling times (t) were as follows:

t +0, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120 hours

The chemistry of the water was monitored throughout the period of study. 'Total' water, pH, conductivity and temperature were determined at each sampling time. 'Filtrable' water samples were taken at daily intervals. Sampling began at 07.45 a.m. 02.08.82.

It was decided to continue sampling for five days since the laboratory study (Sections 2.7, 6.2) suggested that at least 2 - 3 days were needed before a 'levelling off' in barium concentration occurred.

2.84 Analysis

Animals were acid digested as previously described (Sections 2.22, 2.24). The resulting solutions were analysed for Ca, Zn and Ba by atomic absorption spectrophotometry (Section 2.23). Water samples were analysed for Ca, Zn, Ba and Pb.

2.9 PRESENTATION OF RESULTS

2.91 Water

Physico-chemical variables are reported to the number of significant figures and the limit of detection consistent with the method by which they were determined.

Metal concentrations are expressed in mg l^{-1} to the number of decimal places consistent with the sensitivity of the range used for measurement. Calcium is usually measured to two decimal places in the range 0.01 - 10.00mg l^{-1}. However, in this case, except for the preliminary water survey, the values are given to one decimal place.

2.92 Digests

Metal concentrations in animal digests are expressed as µg g^{-1} dry wt using the following conversion from mg l^{-1} digest solution:

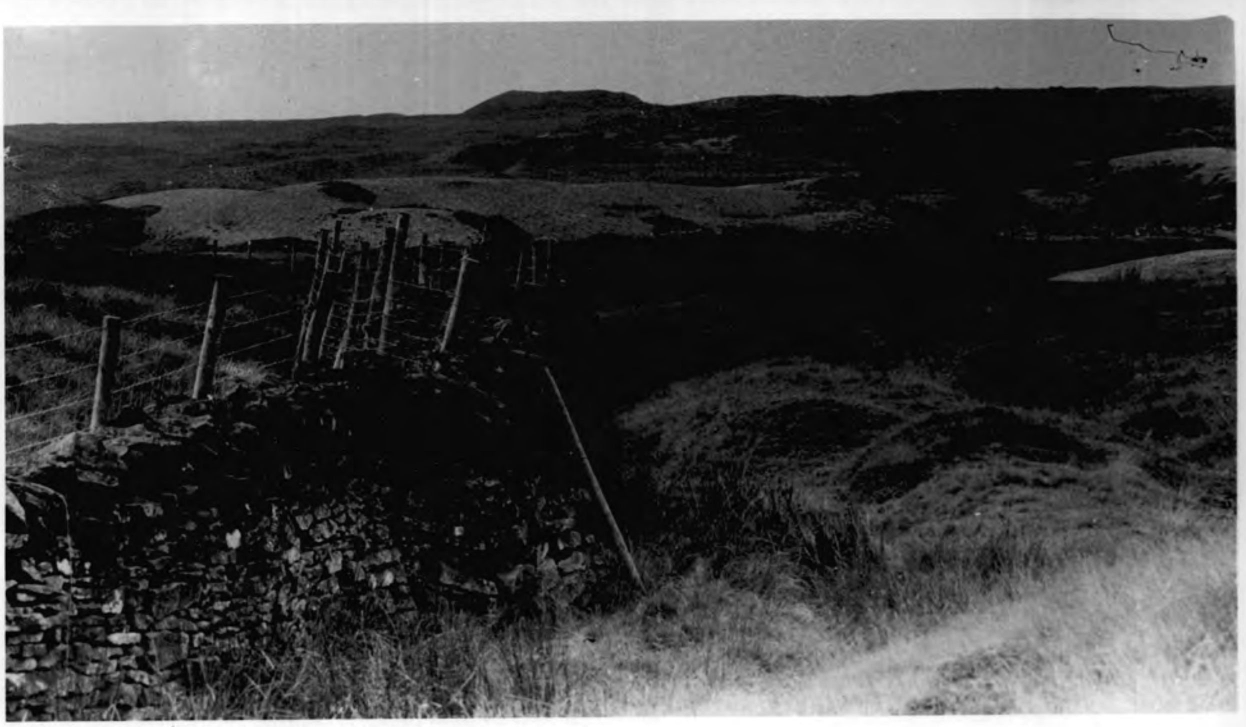
$$\mu g\ g^{-1}\ \text{dry wt} = \frac{mg\ l^{-1} \times \text{volume of digest solution} \times 1000}{\text{dry wt of sample in mg}}$$

The limit of detection of metals in animal samples depends on the amount of sample and the concentration detected in the digest solution. Metal concentrations are reported to the number of significant figures consistent with the limit of detection down to the level of integers. Standard deviations and 95% confidence limits of the mean are treated in the same way. Any exceptions are mentioned in the relevant text. Coefficients of variation are given in percentages to one decimal place.

Figure 2.2a Transplant cage

Figure 2.2b Transplant cage in situ in 'Moor Intake Gill' (0336-40)

Figure 2.3 Fourth Whim tips near Bleaberry Gill (0212), reach 20



CHAPTER 3

THE STUDY AREA

3.1 INTRODUCTION

The study area is situated west of Langthwaite, a small village in Arkengarthdale, N. Yorks (Fig. 3.1) and consists of an area of moorland with disused lead workings and spoil tips (Fig. 2.3).

Bleaberry Gill (0212), the main stream draining this area, arises at 569m and flows approximately south-east for four km. It joins Old Gang Beck (0374) to form Barney Beck (0198), a tributary to the River Swale, entering about three km above Grinton. Bleaberry Gill has a vertical drop of about 230m from its source to its junction with Old Gang Beck. It is surrounded by upland grassland/moorland with occasional exposed waste tips, connected with old mine workings.

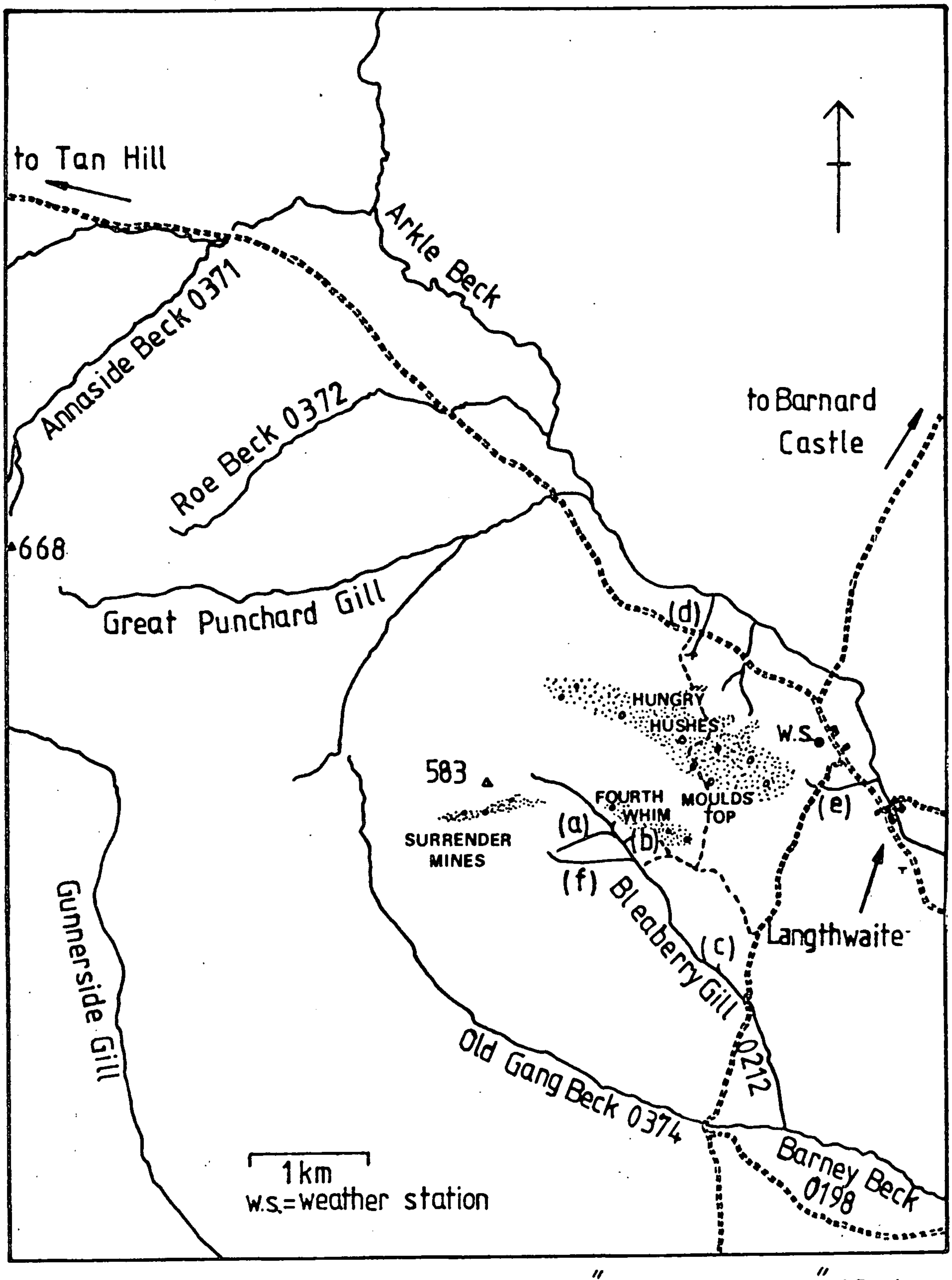
'Mossy Thorn Gill' (0350) is a very small stream of about 40m in length and 0.4m in width. It drains from the base of a hill over a relatively flat area before dropping about 3m to Bleaberry Gill.

'Moor Intake Gill' (0336) is about 0.5km in length and drains in a north-easterly direction to join Arkle Beck. It flows under the road from Langthwaite to Tan Hill. There is little vertical drop above the road; the flow being through upland grassland. Below the road, the stream flows through pasture and the drop increases considerably.

Moulds Old Level (0328) drains a complex of mine tips to join Arkle Beck above Langthwaite.

Old Gang Beck (0374) drains a large mining area to the north and west of Bleaberry Gill.

Annaside Beck (0371) and Roe Beck (0372) drain upland moorland landscape with no evidence of mining activity.



(a) Wetshaw Bottom 0322
(b) "Fourth Whim Sike" 0323
(c) "Mossy Thorn Gill" 0350
(d) "Moor Intake Gill" 0336
(e) Moulds Old Level 0328
(f) "Surrender Moss Gill" 0348

Figure 3.1 The study area

3.2 LOCATION OF SAMPLING SITES

Figs 3.2 and 3.3 give the locations of sampling sites. Sites were defined as 10m lengths of stream, termed reaches (Say *et al.*, 1977). All sites were coded according to the system used at Durham University. In this system each site is given a stream and reach number, e.g. 0212-49

3.21 Surveys of Bleaberry Gill

In a preliminary survey 27 sites were sampled, 20 on Bleaberry Gill, 5 tributaries, Old Gang Beck and Barney Beck (Table 3.1 and Fig. 3.2). From these, 9 reaches were chosen to be sampled in more detail (Fig. 3.3). These were:

0212 - 20 - 24 - 36 - 46 - 55 - 99
0322 - 99
0350 - 50
0374 - 99

0212-49 was included in the study of the water chemistry of Bleaberry Gill.

3.22 Investigation of metals in *Baetis* sp(p). and water

Sites were chosen to include a wide range of aqueous metal concentrations, with particular regard to barium. The following sites were used; for locations see Table 3.1 and Fig. 3.3:

0212 - 20 - 24 - 36 - 45 - 46 - 49 - 99
0323 - 99
0350 - 50
0336 - 50 - 85
0371 - 98
0372 - 60
0328 - 10
0374 - 85
0008 - 65

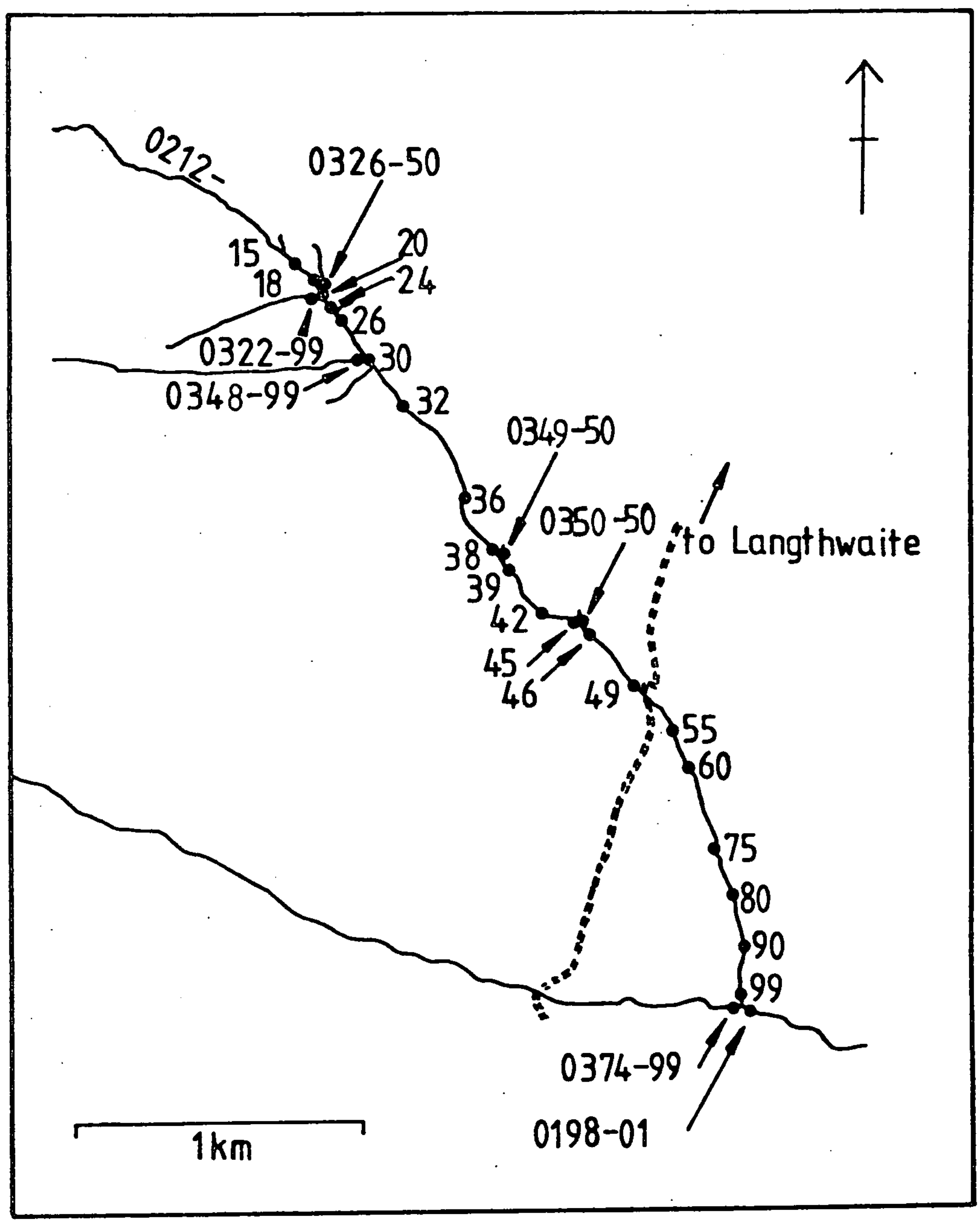


Figure 3.2 Sample sites, on Bleaberry Gill and associated tributaries, used in preliminary survey 27.04.82

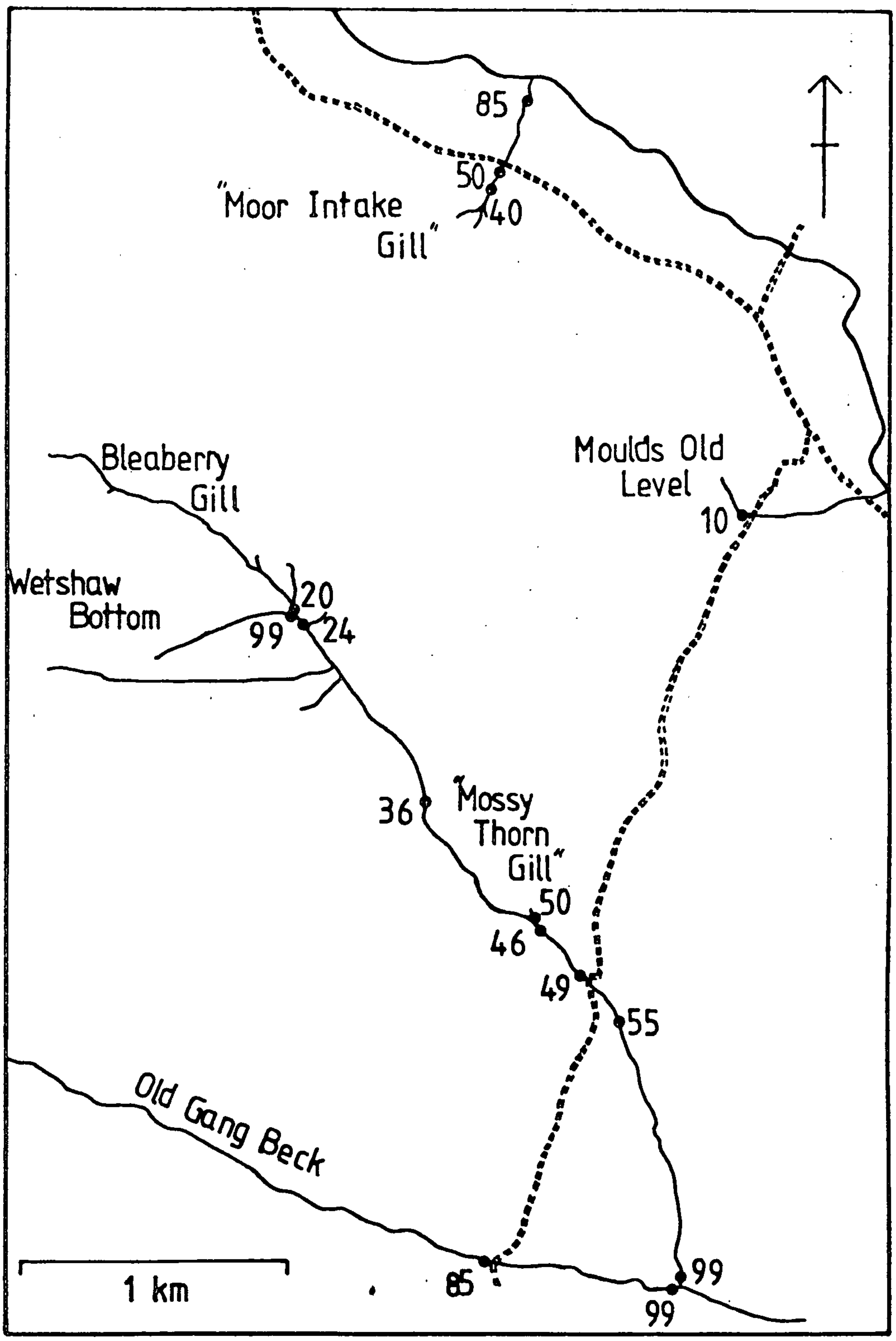


Figure 3.3 Sample sites used in:

a) detailed survey of Bleaberry Gill
b) investigation of metals in *Baetis* sp(p). and water
c) transplant studies on metal uptake by *G. pulex*

Sites used in Section 3.21

stream - reach			grid reference	notes
0212	-	15	NY 981024	Bleaberry Gill
0212	-	18	NY 981023	Bleaberry Gill
0212	-	20	NY 981023	Bleaberry Gill above Wetshaw Bottom
0212	-	24	NY 982022	Bleaberry Gill below junction with Wetshaw Bottom
0212	-	26	NY 982022	Bleaberry Gill below junction with 'Fourth Whim Sike' (0323)
0212	-	30	NY 983021	Bleaberry Gill below entry of 'Surrender Moss Gill' (0348)
0212	-	32	NY 984019	Bleaberry Gill
0212	-	36	NY 986016	Bleaberry Gill
0212	-	38	NY 987014	Bleaberry Gill above entry of 0349
0212	-	39	NY 987014	Bleaberry Gill below entry of 0349
0212	-	42	NY 989012	Bleaberry Gill
0212	-	45	NY 990012	Bleaberry Gill above entry of 0350
0212	-	46	NY 990012	Bleaberry Gill below entry of 0350
0212	-	49	NY 992010	Bleaberry Gill
0212	-	55	NY 994008	Bleaberry Gill
0212	-	60	NY 994007	Bleaberry Gill
0212	-	75	NY 995004	Bleaberry Gill
0212	-	80	NY 996002	Bleaberry Gill
0212	-	90	NY 996000	Bleaberry Gill
0212	-	99	SD 996999	Bleaberry Gill
0326	-	50	NY 981023	'Fourth Whim Gill' tributary to Bleaberry Gill
0322	-	99	NY 981022	Wetshaw Bottom tributary to Bleaberry Gill
0348	-	99	NY 982021	'Surrender Moss Gill' tributary to Bleaberry Gill
0349	-	50	NY 987014	'Mossy Thorn Seepage' tributary to Bleaberry Gill'
0350	-	50	NY 990012	'Mossy Thorn Gill' tributary to Bleaberry Gill
0374	-	99	SD 995998	Old Gang Beck
0198	-	01	SD 996998	Barney Beck

Other sites used in Sections 3.22, 3.23

0328	-	10	NY 998026	Moulds Old Level
0374	-	85	SD 988999	Old Gang Beck, reach situated above Surrender Bridge
0336	-	40	NY 989038	'Moor Intake Gill' reach just above road
0336	-	50	NY 989039	'Moor Intake Gill' reach about 50m above road
0336	-	85	NY 990041	'Moor Intake Gill' reach about 150m below road
0323	-	99	NY 982022	'Fourth Whim Sike' tributary to Bleaberry Gill
0371	-	98	NY 946070	Annaside Beck
0372	-	60	NY 968057	Roe Beck
0008	-	65	NZ 287410	River Wear upstream of bridge at Shincliffe

Table 3.1 Location of sampling sites

3.23 Transplant studies on metal uptake by *G. pulex*

G. pulex individuals were removed from 'Mossy Thorn Gill' (0350-50) for the laboratory study (Section 6.2) and were transferred from 0350-50 to 'Moor Intake Gill' (0336-40) in the field transplant study (Section 6.3).

3.3 DESCRIPTION OF SITES USED IN DETAILED SURVEY OF BLEABERRY GILL

Table 3.2 gives a description of the reaches used in the detailed survey of Bleaberry Gill (0212) on 10.05.82 (Section 4.2). The type of substrate present is one of the main factors affecting the species composition of the invertebrate fauna. The stream sampling reaches are shown in Fig. 3.4a-h.

stream - reach	width (range) m	site description
0212 - 20	0.2 - 0.5	Very shallow 20cm, depositing substrate with silt and gravel
0212 - 24	0.5 - 1.5	Depth 20cm, substrate of bedrock and gravel, iron oxide deposit, fairly eroding substrate
0212 - 36	1.0 - 2.5	Mixed substrate within one reach, good depositing and eroding areas, large boulders, stones, gravel and silt. Depth 0.2 - 0.6m. Good filamentous algal growth, covered in iron oxide deposit
0212 - 46	2.0 - 3.0	Some areas of deposition of gravel but mostly eroding substrate with large stones. Depth 0.2 - 0.4m
0212 - 49	2.0 - 3.0	Some areas of deposition of sediment, mostly eroding with a stony bed
0212 - 55	2.0 - 3.0	Eroding substrate, few boulders, mostly large stones, some areas of deposition of sand and gravel. Depth 0.2 - 0.3m
0212 - 99	2.0 - 4.0	Very eroding substrate, many large boulders, very little gravel and silt. Depth 0.2 - 0.8m
0322 - 99	0.2 - 0.5	Small stream flowing through peat, iron oxide deposits
0350 - 50	0.3 - 0.5	Small stream draining from the base of a hill, some depositing and eroding sections, good moss growth on stones, mostly silt and gravel bed. Depth 0.10 - 0.15m
0374 - 99	3.0 - 5.0	Very eroding substrate. Stream experiences flash floods. Very little algal and moss growth. Large boulders and stony bed

N.B. 10.05.82 flow rate 2

Table 3.2 Description of reaches used in detailed Bleaberry Gill survey

Figure 3.4a Bleaberry Gill (0212) reach 20, looking upstream

Figure 3.4b Bleaberry Gill (0212) reach 24, looking downstream

Figure 3.4c Bleaberry Gill (0212), reach 36, looking upstream

Figure 3.4a-h Stream sampling reaches

Figure 3.4d Bleaberry Gill (O212), reach 46, looking upstream

Figure 3.4e Bleaberry Gill (O212), reach 49, looking upstream

Figure 3.4f Bleaberry Gill (O212), reach 55, looking upstream

Figure 3.4 (continued) Stream sampling reaches

Figure 3.4g Bleaberry Gill (0212) (from left), reach 99, joining Old Gang Beck (from right) (0374), reach 99, to form Barney Beck (0198)

Figure 3.4h 'Mossy Thorn Gill' (0350), reach 50, looking upstream

Figure 3.4 (continued) Stream sampling reaches

3.4 METEOROLOGY

Information about the area was obtained from the Newcastle Meteorology office. General information about the climate of the area is given in Section 1.31 and Table 1.1.

In this study rainfall was an important factor as it affected the flow of the streams, with subsequent changes in their aqueous metal concentrations. Daily rainfall data were obtained for a weather station near Langthwaite (Fig. 3.1 W.S.). The data are shown in Table 3.4 and Fig. 3.5.

The monthly totals are compared, in Table 3.3, with average monthly rainfall data for the Upland Zone of the Northern Pennines (east of the watershed and north of the Aire gap) obtained from the Ministry of Agriculture, Fisheries and Food. Technical Bulletin 35 (Smith, 1976).

Month	average monthly rainfall 1941 - 1970 (mm)	monthly rainfall 1982 (mm)
Jan	99	129.2
Feb	72	59.7
Mar	63	139.2
Apr	67	26.5
May	70	55.3
June	71	108.3
July	85	33.8
Aug	108	100.3
Sept	111	
Oct	106	
Nov	107	
Dec	109	

average annual rainfall 1068mm

Table 3.3 Comparison between the average monthly rainfall (1941-70) and the actual totals for 1982

DAY NO.	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG
1	0.0	0.2	4.1	0.5	0.5	0.1	0.0	0.2
2	25.6	0.2	15.4	0.0	26.4	0.0	0.3	0.0
3	28.7	0.0	7.2	1.2	1.3	0.0	0.0	0.0
4	25.9	1.8	0.0	0.0	0.2	0.0	0.7	5.4
5	10.5	6.3	0.9	3.5	0.0	0.4	1.9	0.7
6	0.0	2.6	7.0	3.0	0.0	3.6	0.0	0.0
7	0.0	3.4	0.0	9.1	0.0	0.0	0.0	0.0
8	5.1	11.4	1.3	0.1	0.0	13.2	0.0	0.0
9	2.0	0.8	20.7	0.7	0.0	2.3	4.4	0.0
10	0.0	0.6	9.6	0.0	0.0	2.3	0.0	0.0
11	0.0	0.9	13.7	0.0	0.0	0.7	0.0	0.4
12	0.0	4.5	5.0	0.0	0.0	4.9	0.0	0.4
13	0.0	6.6	4.9	0.0	0.0	0.4	0.0	1.6
14	0.0	0.0	24.5	0.0	0.0	0.0	9.9	12.9
15	0.0	0.0	6.2	0.0	0.9	6.9	15.0	2.8
16	0.4	0.8	3.7	0.3	0.0	0.1	0.0	5.7
17	0.4	1.4	1.1	0.0	0.8	0.0	0.0	19.7
18	0.1	0.0	0.5	0.0	0.0	4.5	0.0	4.6
19	1.4	1.3	8.5	0.0	0.0	1.4	0.0	11.3
20	6.0	0.0	0.8	0.0	0.0	0.8	0.0	3.9
21	5.8	0.0	0.1	0.0	6.3	7.7	0.0	2.6
22	3.9	0.0	0.0	0.0	5.6	21.9	0.0	1.4
23	0.0	0.0	0.0	0.0	2.5	1.2	0.0	0.7
24	0.1	0.1	0.0	0.0	2.9	1.3	0.0	6.4
25	6.9	3.3	0.0	0.0	1.3	17.7	0.0	3.5
26	2.2	4.1	0.0	0.0	0.0	10.3	0.0	1.6
27	0.2	0.0	0.0	0.0	6.6	2.7	0.0	3.3
28	1.7	9.4	0.0	0.8	0.0	1.4	0.0	0.0
29	0.0	-	0.9	0.3	0.0	0.0	0.2	8.4
30	2.1	-	0.0	7.0	0.0	2.5	0.4	2.8
31	0.2	-	3.1	-	0.0	-	1.0	0.0
Totals mm	129.2	59.7	139.2	26.5	55.3	108.3	33.8	100.3

Annual average mm 1115.0

Table 3.4 Daily rainfall (mm) for 1982 up to 31 August - Arkengarthdale

Notes
(1) Daily totals relate to 24-hour periods commencing at 0900 GMT on the day of entry
(2) Falls of less than 0.05mm are shown as 0.0
(3) Station no. 051684
(4) National grid reference Easting 3999 Northing 5030
(5) Altitude 294m A.S.M.L.

The average annual rainfall for the Arkengarthdale weather station (No.051684) is 1115.0 (Table 3.4). This is not substantially different from that of the Upland Zone of the Northern Pennines.

It can be concluded from Table 3.4 that:

i) January, March and June 1982 were relatively wet months
ii) February and August were near average rainfall
iii) April and July were relatively dry months

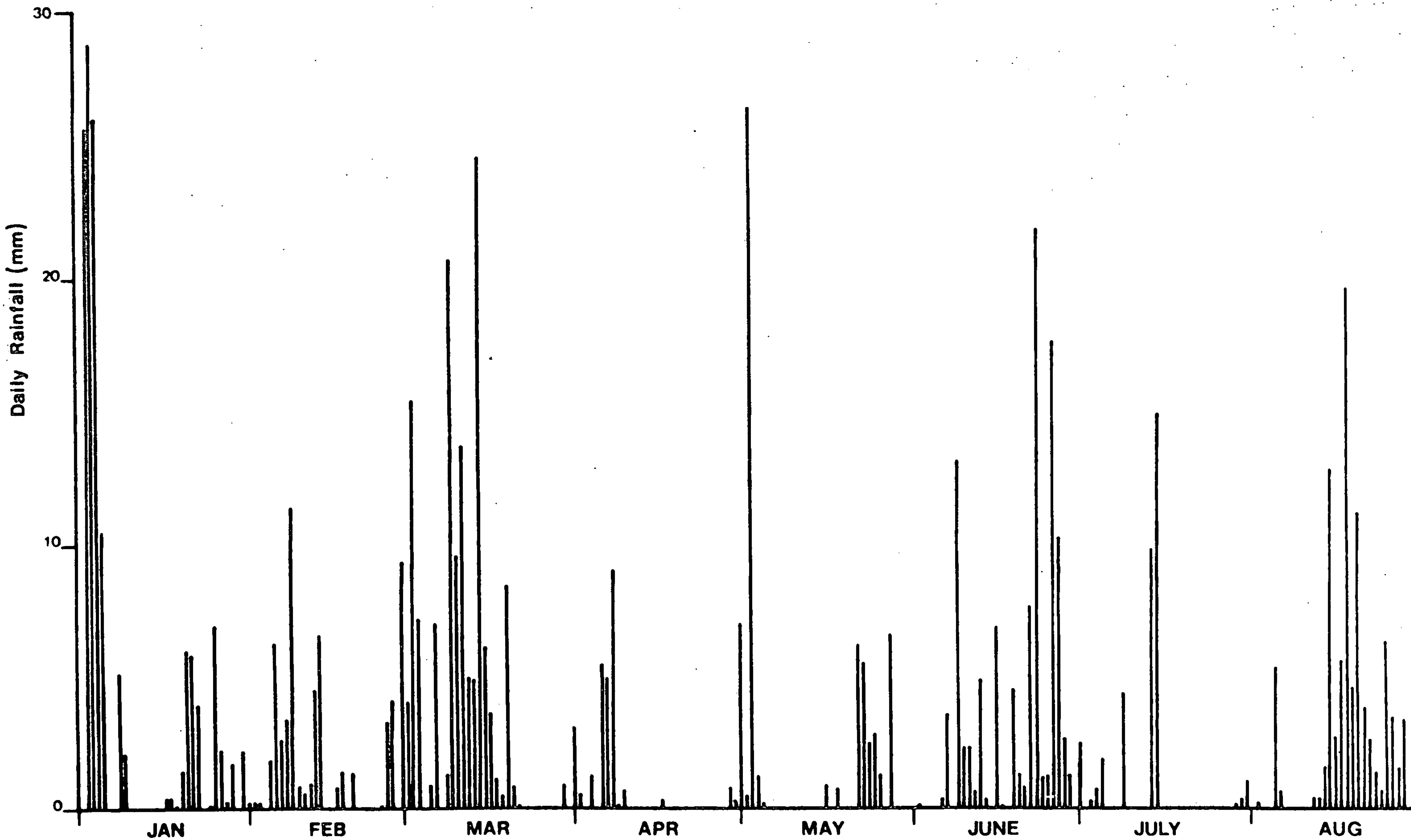


Figure 3.5 Daily rainfall (mm) for 1982 up to 31 August - Arkengarthdale

3.5 PREVIOUS WORK ON THE STUDY AREA

A preliminary study was carried out on the water, sediments and biota of the area (Say and Whitton, 1982). Some of this data for January and March 1982 has been included in the results of this study.

3.6 CHANGES IN THE AREA DURING THE PRESENT STUDY

3.61 Flow

It was hoped to obtain information on the flow of the River Swale from a gauging station situated near Richmond. However, this station no longer measures flow and therefore it was not possible to obtain flow values which could have been related to the state of Bleaberry Gill and the other streams during the study period.

In general the streams were in a state of fairly low flow (flow rate 2). There was little or no surface run-off from the surrounding moorland or grassland. The flow did increase considerably on some occasions after heavy rain (flow rate 4 - 5). It is likely that there was more variation in stream flow than was noted in the short periods of time spent in the study area. The rainfall data, in Fig. 3.5 and Table 3.4, show large variations throughout the study period.

Flow was very low at the beginning of the study (flow rate 1), 27.04.82, after a very dry April. 'Fourth Whim Sike' (0323), a high barium tributary of Bleaberry Gill, was dry on this occasion; on all previous sampling occasions it had been flowing (Say and Whitton, 1982). This stream was dry on all subsequent occasions except 24.06.82 after persistent rain on 22.06.82.

The majority of the water in Bleaberry Gill during low flow was draining the moorland blanket peat. Input was particularly high from Wetshaw Bottom (0322) and 'Surrender Moss Gill' (0348).

3.62 Mining operations

There was no mining activity during the period of study. The mining company remade a track up to Moulds Top, from the north, in July (Fig. 3.1), and, during the beginning of August, some preparatory work was carried out in the Moulds Top/Fourth Whim area.

3.7 PRESENT MINING DEVELOPMENTS

The proposed development, in the area of Bleaberry Gill, is for the extraction of barite, fluorite and galena from existing mine waste tips. The operating company hopes to remove 30000 tonnes of material from the Fourth Whim tips (Figs. 2.3, 3.1) which consist of 48.3% barite, 15.2% fluorite, 7.1% witherite, 1.5% lead and 0.5% zinc; plus 150000 tonnes of material brought in from other parts of Arkengarthdale. Wet gravity processing will be conducted on site. A series of settling ponds will be filled initially with water abstracted from Bleaberry Gill below Wetshaw Bottom (0322). The plant will recycle water and, under normal conditions, there will be no discharge to Bleaberry Gill. The plant is expected to lose up to 10% water volume per week through percolation, evaporation and in concentrates removed from the site. Once in operation, the plant water will probably become saturated with metals. Should the pond impoundment fail or the processing water be flushed out of the plant by a flood, then Bleaberry Gill may be expected to receive a discharge of such contaminated water. Movement of the tips in the area of Fourth Whim may also alter drainage patterns and increase the heavy metal concentrations in Bleaberry Gill.

CHAPTER 4

SURVEYS OF BLEABERRY GILL

A limited survey had been carried out over the five months from November to March (Say and Whitton, 1982). This provided introductory data to the springs and streams of the present study area, particularly Bleaberry Gill. However, since only three sites were studied on Bleaberry Gill, it was decided that more work should be carried out to give a clearer picture of its nature.

4.1 PRELIMINARY SURVEY

This was carried out to establish sites for sampling in more detail. 27 sites were sampled on 27.04.82 along Bleaberry Gill and its associated tributaries (Fig. 3.2, Section 3.21). 'Total' water samples only were taken at each reach; this established the general nature of the water chemistry. Samples were analysed for Ca, Fe, Zn, Ba and Pb by atomic absorption spectrophotometry (Section 2.23). Results are shown in Table 4.1. Figs 4.1a-e are included to illustrate them more clearly.

Comments

i) Concentrations of Ca, Fe, Zn, Ba and Pb in 'total' water samples varied substantially along the length of Bleaberry Gill

ii) Tributaries had a very marked effect on the metal concentrations in Bleaberry Gill. Calcium, iron and barium concentrations changed noticeably below the tributaries

iii) Zinc levels were relatively high at the top of Bleaberry Gill but then dropped to a fairly steady level between reach 20 and 24

iv) Lead concentrations did not relate directly to the input from tributaries. This suggests that the levels of lead are influenced to a greater extent by substrate or ground water seepage into the gill

v) In three cases barium concentrations increased after the rapid decrease which followed inputs from streams with low barium. This would suggest that barium is taken into solution from the substrate or by ground water seepage

vi) Old Gang Beck (0374) at reach 99 had a relatively high barium concentration similar to that at 0212-20. It also had substantially higher calcium, zinc and lead concentrations compared with Bleaberry Gill

4.2 DETAILED SURVEY

Nine sites were chosen to be studied in more detail (Section 3.21). Six sites on Bleaberry Gill were chosen to show the variation in the stream (aqueous metal concentrations, substrate and flow) over its 230m drop. 0212-49 was included in the water chemistry study for comparison with results obtained by Say and Whitton (1982). Table 3.2 and Figs 3.4a-h give descriptions of these sites. The tributaries were included as they provide input of a very different water quality. Old Gang Beck was included for comparison with Bleaberry Gill; it had much higher aqueous zinc and lead and relatively high barium and calcium.

This survey was carried out over two days. The fauna was collected on 10.05.82 and the water chemistry on 14.05.82. No attempt was made to correlate the metal compositions of the water and animals at this stage. This was a descriptive survey to provide information on the general characteristics of Bleaberry Gill.

stream reach	Ca	Fe	Zn	Ba	Pb
0212 - 15	17.1	1.66	0.108	0.50	0.034
0212 - 18	15.1	1.82	0.085	0.44	0.067
0212 - 20	19.9	0.67	0.105	1.58	0.048
0212 - 24	-	1.19	0.045	0.45	0.034
0212 - 26	4.44	1.15	0.045	0.74	0.023
0212 - 30	12.2	7.14	0.043	0.70	0.021
0212 - 32	12.8	4.82	0.038	0.72	0.029
0212 - 36	12.3	2.31	0.038	0.64	0.044
0212 - 38	8.70	1.12	0.040	0.70	0.058
0212 - 39	6.20	0.59	0.043	0.47	0.031
0212 - 42	8.60	0.57	0.030	0.82	0.037
0212 - 45	9.90	0.49	0.033	0.88	0.034
0212 - 46	23.2	0.33	0.035	0.52	0.028
0212 - 49	22.1	0.26	0.035	0.69	0.027
0212 - 55	23.0	0.21	0.043	0.77	0.023
0212 - 60	25.2	0.19	0.035	0.72	0.021
0212 - 75	26.4	0.13	0.050	0.78	0.040
0212 - 80	28.8	0.11	0.045	0.72	0.022
0212 - 90	28.6	0.11	0.036	0.80	0.043
0212 - 99	29.2	0.08	0.035	0.72	0.042
0326 - 50	37.3	0.77	0.490	3.25	0.113
0322 - 99	1.92	1.30	0.033	0.16	0.011
0348 - 99	18.9	11.2	0.014	0.33	0.007
0349 - 50	4.80	0.05	0.050	0.26	0.006
0350 - 50	45.0	0.05	0.030	0.34	0.047
0374 - 99	55.0	0.04	0.145	1.52	0.116
0198 - 01	46.4	0.04	0.130	1.29	0.093

Table 4.1 Preliminary survey 27.04.82. Aqueous metals (mg l^{-1}) in 'total' water samples from Bleaberry Gill and associated tributaries. Table 3.1 and Fig. 3.2 give stream and reach locations

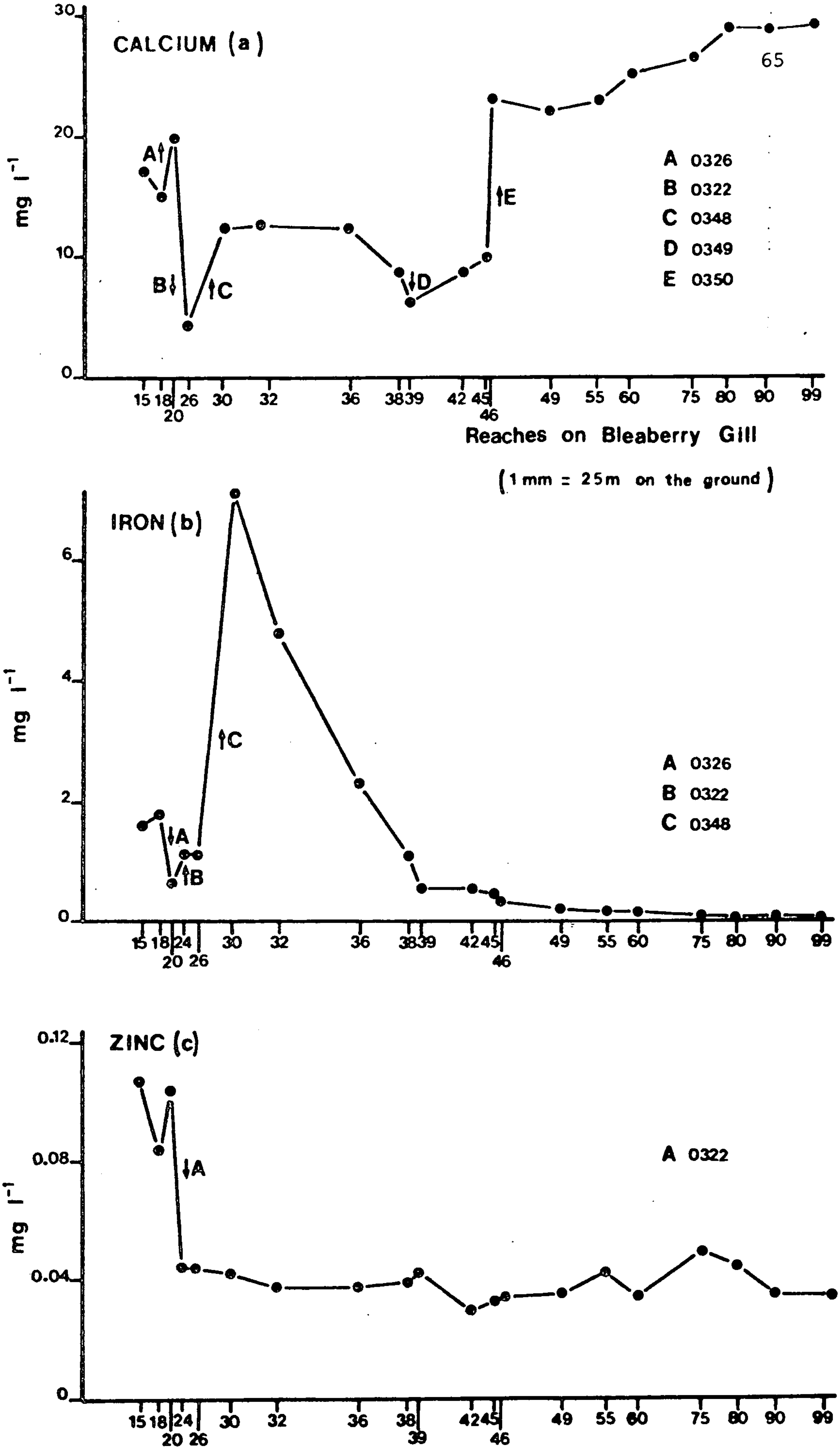


Figure 4.1a-c Calcium, iron and zinc in 'total' water (mg l^{-1}) from 20 reaches on Bleaberry Gill (0212), 27.04.82. Fig. 3.2 gives reach locations. Arrows indicate input from tributaries which affected the aqueous metal concentration substantially

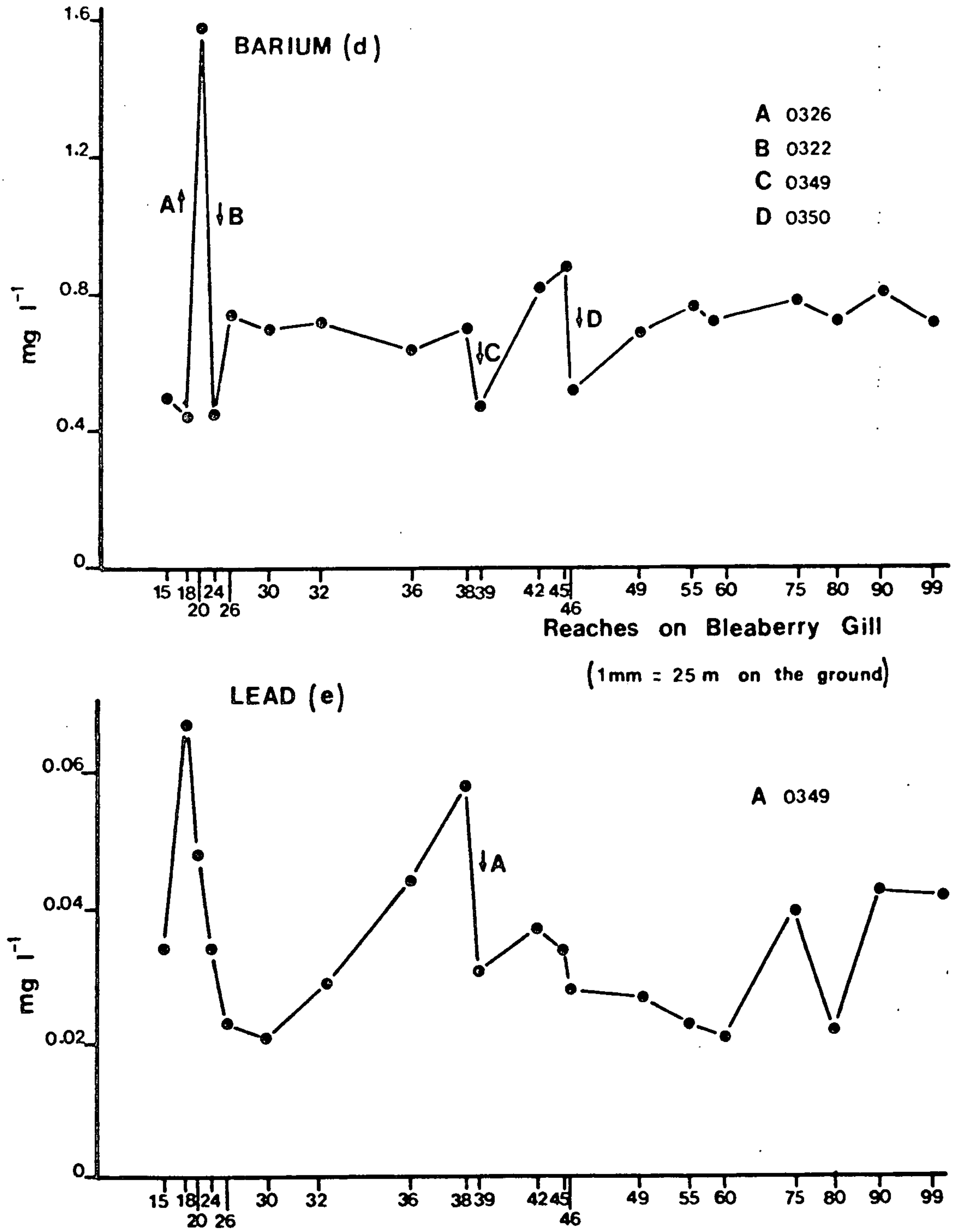


Fig. 4.1d-e Barium and lead in 'total' water (mg l^{-1}) from 20 reaches on Bleaberry Gill (0212), 27.04.82. Fig. 3.2 gives reach locations. Arrows indicate input from tributaries which affected the aqueous metal concentration substantially

4.21 Water chemistry

'Total' and 'filtrable' water samples (Table 4.2) were collected at each reach as described above (Section 2.31). pH and conductivity were determined (Section 2.321). Water samples were also collected for sulphate analysis (Sections 2.31, 2.322).

Comments

i) Metal concentrations were similar to those found in the preliminary survey (Section 4.1)

ii) There was little difference between 'total' and 'filtrable' calcium, zinc and barium, but iron and lead 'total' and 'filtrable' concentrations were substantially different

iii) Conductivity and pH increased downstream from 0212-24. 0212-20 had much higher values for these variables than 0212-24

iv) Sulphate - S levels were approximately constant along Bleaberry Gill. Input from Wetshaw Bottom (0322) reduced the concentration at 0212-24.

v) 'Mossy Thorn Gill' (0350) and Old Gang Beck (0374) had comparatively high conductivity, sulphate - S and calcium concentrations

4.22 Flora

The flora of Bleaberry Gill was outside the scope of this study. A parallel study was carried out at the same time by Owen, 1983.

stream	reach	conductivity μScm^{-1}	pH	SO_4^{2-} - S	Ca		Fe		Zn		Ba		Pb	
					T	F	T	F	T	F	T	F	T	F
0212 -	20	121	6.6	3.2	20.2	19.7	0.45	0.23	0.108	0.103	2.00	2.00	0.032	0.012
0212 -	24	59	5.8	2.5	4.0	4.3	1.11	0.32	0.048	0.039	0.53	0.49	0.016	0.009
0212 -	36	99	6.5	2.8	9.8	9.8	1.30	0.30	0.047	0.032	0.78	0.78	0.043	0.019
0212 -	46	154	7.3	3.3	20.5	20.5	0.25	0.12	0.038	0.037	0.63	0.59	0.019	0.011
0212 -	49	153	7.1	3.2	21.6	21.5	0.25	0.18	0.042	0.045	0.78	0.73	0.020	0.011
0212 -	55	156	7.5	3.5	22.1	22.1	0.27	0.12	0.045	0.037	0.86	0.82	0.029	0.011
0212 -	99	195	7.6	3.5	30.3	30.1	0.13	0.10	0.051	0.049	1.10	1.08	0.018	0.009
0322 -	99	49	5.8	2.5	1.6	1.7	1.18	0.74	0.047	0.037	0.14	0.12	0.008	0.008
0350 -	50	285	7.7	5.1	47.9	47.8	<0.03	<0.03	0.033	0.037	0.39	0.31	0.008	0.004
0374 -	99	292	7.2	7.1	57.5	57.6	<0.03	<0.03	0.181	0.181	1.84	1.80	0.078	0.067

Table 4.2 Selected water chemistry variables for reaches on Bleaberry Gill (0212), Wetshaw Bottom (0322), 'Mossy Thorn Gill' (0350) and Old Gang Beck (0374), sampled on 14.05.82. Fig. 3.3 gives stream and reach locations

(metal and SO_4^{2-} - S concentrations in mg l^{-1}; T = 'total', F = 'filtrable')

4.23 Fauna

The invertebrate fauna was sampled at each reach, with 5 minute kick samples taken to include as many microhabitats as possible. Samples were sorted and identified in the laboratory (Section 2.41). The results of this survey are shown in Table 4.3. Although accurate quantitative measures of invertebrate stream animals are very difficult to obtain, it was considered useful to give some estimate of their abundance on a scale of 1 - 4. This estimate should be treated with caution and is presented to give more information than simple presence/absence. Not all organisms were identified to the same level; some were identified to species, some to genus and some to family.

Comments

i) The invertebrate fauna consisted almost entirely of insects. These were mostly aquatic nymph and larval forms, with a few adult beetles and the bug *Velia* sp. Where they occurred, other groups were represented by few individuals. Such groups were the Tricladida, Mollusca, Hydracarina and Malacostraca (*G. pulex* did occur in large numbers at 0350-50 'Mossy Thorn Gill')

ii) The fauna of Bleaberry Gill was dominated numerically by the Plecoptera, particularly the genus *Nemoura*, towards the top of Bleaberry Gill, and *Leuctra inermis*

iii) The Plecoptera (*Leuctra inermis, Amphinemura sulcicollis, Chloroperla torrentium)* and the Chironomidae were present at all sites on Bleaberry Gill

iv) The Ephemeroptera were relatively well represented, being found at five out of the six sites on Bleaberry Gill

	Invertebrate taxon	stream reach	0212 - 20	0212 - 24	0212 - 36	0212 - 46	0212 - 55	0212 - 99	0322 - 99	0350 - 50	0374 - 99
Turbellaria Tricladida					+					+	
Gastropoda	*Potamopyrgus jenkinsi* (Smith)									1	
Bivalvia	*Pisidium* sp.									1	
Hydracarina				1	1	2	2	2		1	1
Malacostraca	*Gammarus pulex* (L.)				2	2				4	
Ephemeroptera	*Ameletus inopinatus* Eaton				2	1	1				
	Baetis sp(p).		1		1	2	1	2		2	2
	Rhithrogena semicolorata (Curtis)					1	1	4			2
	Heptagenia lateralis (Curtis)					2	2	1		1	1
	Caenis sp.							1			
Plecoptera	*Brachyptera risi* (Morton)			1	2	1	1	1			
	Protonemura sp(p).			1	2						
	Amphinemura sulcicollis (Stephens)		2	1	2	2	2	2	1	1	1
	Nemoura sp(p).		4	4	2	1		1	2	4	
	Leuctra inermis Kempny		1	2	3	4	4	3	1	2	2
	Leuctra hippopus (Kempny)		1	1							
	Leuctra nigra (Olivier)		1		1	1				2	
	Isoperla grammatica (Poda)				1	1	1	2			
	Dinocras cephalotes (Curtis)							2			
	Chloroperla torrentium (Pictet)		1	2	2	2	2	1	1		
	Chloroperla tripunctata (Scopoli)							1			
Hemiptera	*Velia* sp.								1		
Coleoptera	Dytiscidae		2	1		2	2	1	1	1	
	Helodidae				1					1	
	Elminthidae		3			1	1	3		1	1
Megaloptera	*Sialis fuliginosa* Pictet		1								
Trichoptera	*Rhyacophila dorsalis* (Curtis)				1			1		1	
	Agapetus sp.				2					4	
	Plectrocnemia conspersa (Curtis)		3	1		1	1		1	1	
	Hydropsyche instabilis (Curtis)							1			
	Hydroptilidae							1			1
	Limnephilidae		1	1	1				1	3	
Diptera	Tipulidae *Pedicia rivosa* (L.)		+								
	Dicranota sp(p).						+		+	+	
	Limnophila cf. *verralli*					+	+		+		
	Psychodidae cf. *Pericoma* sp.				+					+	
	Chironomidae		+	+	+	+	+	+	+	+	
	Simuliidae		2	4	3		1		4	3	2
	Empididae cf. *Hemerodromia* sp.			+					+	+	
		Taxa	15	14	20	18	17	19	13	23	9

Abundance Scale 1 1 - 5 individuals/sample
2 6 - 20 ,,
3 21 - 50 ,,
4 >50
+ present with no abundance estimate

Table 4.3 Invertebrates collected from Bleaberry Gill (O212) (five sites), Wetshaw Bottom (O322), 'Mossy Thorn Gill' (O350) and Old Gang Beck (O374), 10.05.82. Fig. 3.3 gives stream and reach locations

v) The Trichoptera were represented at each site, though frequently by few individuals. *Plectrocnemia conspersa* was particularly abundant at 0212-20

vi) The Simuliidae were most abundant in the three upper reaches of Bleaberry Gill

vii) *Sialis fuliginosa* was found only at 0212-20

viii) *Caenis* sp., *Chloroperla tripunctata* and *Dinocras cephalotes* were found only at 0212-99

ix) *Gammarus pulex* was found at two reaches on Bleaberry Gill, above and below the entry of 'Mossy Thorn Gill' (0350)

x) 'Mossy Thorn Gill' (0350) maintained the most taxa, including large numbers of *G. pulex*, *Nemoura* sp(p.), *Agapetus* sp., Limnephilidae and Simuliidae. This was the only site studied that supported molluscs

xi) Wetshaw Bottom (0322) and Old Gang Beck (0374) were poorly represented by invertebrates, in both species number and abundance

There are many problems with sampling in fresh water and it is possible that the sampling method used in this study may not have revealed the presence of certain taxa. This problem is discussed further in Section 7.221.

4.3 VARIATION IN AQUEOUS METAL CONCENTRATIONS AT SELECTED REACHES

Several of the reaches used in the investigations of Chapters 4, 5 and 6 were sampled more than once. The results obtained give some indication of the variation in aqueous metal concentrations experienced by the fauna and flora of Bleaberry Gill and two other streams.

4.31 Results

Data were collected on 'total' aqueous concentrations of Ca, Zn, Ba and Pb at seven sites (Table 4.5). All reaches shown were sampled three or more times. Values obtained on 27.01.82 and 16.03.82 by Say and Whitton, 1982 and on 17.06.82 by Owen, 1983 are also included. The ranges of concentrations given in Table 4.5 are summarized in Table 4.4.

stream reach	n	Ca	Zn	Ba	Pb
0212 - 20	7	8.8 - 21.2	0.105 - 0.26	1.14 - 2.48	0.032 - 0.217
0212 - 24	4	4.0 - 8.1	0.045 - 0.202	0.45 - 1.37	0.016 - 0.044
0212 - 36	3	8.9 - 12.3	0.038 - 0.047	0.64 - 0.78	0.043 - 0.069
0212 - 46	4	9.0 - 23.2	0.027 - 0.166	0.52 - 1.00	0.019 - 0.076
0212 - 49	11	6.6 - 22.1	0.031 - 0.219	0.51 - 1.00	0.020 - 0.090
0350 - 50	5	29.1 - 47.9	0.011 - 0.033	0.33 - 0.43	0.007 - 0.047
0336 - 50	3	28.1 - 72.7	0.103 - 0.27	3.15 - 27.1	0.005 - 0.034

Table 4.4 Ranges of 'total' aqueous metals (mg l^{-1}) in Bleaberry Gill (0212) (five sites), 'Mossy Thorn Gill' (0350) and 'Moor Intake Gill' (0336)

stream reach	date														
	27.01	16.03	27.04	14.05	19.05	01.06	17.06	23.06	24.06	28.06	04.07	31.07	03.08	04.08	05.08
Calcium															
0212 - 20	8.8	9.3	19.9	20.2	17.5	-	-	-	14.8	-	-	-	21.2	-	-
0212 - 24	-	-	-	4.0	-	-	-	-	8.1	-	-	-	5.2	-	-
0212 - 36	-	-	12.3	9.8	-	8.9	-	-	-	-	-	-	-	-	-
0212 - 46	-	-	23.2	20.5	-	17.4	-	-	9.0	-	-	-	-	-	-
0212 - 49	8.3	7.0	22.1	21.6	16.2	-	16.4	6.6	8.8	8.99	13.5	-	-	17.3	-
0350 - 50	-	-	45.0	47.9	-	-	-	-	29.1	-	-	32.3	-	32.5	-
0336 - 50	-	28.1	-	-	-	-	-	-	-	42.4	-	-	-	-	72.7
Zinc															
0212 - 20	0.165	0.160	0.105	0.108	0.109	-	-	-	0.26	-	-	-	0.134	-	-
0212 - 24	-	-	0.045	0.048	-	-	-	-	0.202	-	-	-	0.050	-	-
0212 - 36	-	-	0.038	0.047	-	0.047	-	-	-	-	-	-	-	-	-
0212 - 46	-	-	0.035	0.038	-	0.027	-	-	0.166	-	-	-	-	-	-
0212 - 49	0.095	0.088	0.035	0.042	0.031	-	0.070	0.219	0.122	0.124	0.063	-	-	0.034	-
0350 - 50	-	-	0.030	0.033	-	-	-	-	0.032	-	-	0.015	-	0.011	-
0336 - 50	-	0.25	-	-	-	-	-	-	-	0.27	-	-	-	-	0.103
Barium															
0212 - 20	1.14	1.43	1.58	2.00	2.12	-	-	-	2.30	-	-	-	2.48	-	-
0212 - 24	-	-	0.45	0.53	-	-	-	-	1.37	-	-	-	0.62	-	-
0212 - 36	-	-	0.64	0.78	-	0.77	-	-	-	-	-	-	-	-	-
0212 - 46	-	-	0.52	0.63	-	0.59	-	-	1.00	-	-	-	-	-	-
0212 - 49	0.51	0.66	0.69	0.78	0.88	-	0.78	0.87	0.94	1.00	0.79	-	-	0.72	-
0350 - 50	-	-	0.34	0.39	-	-	-	-	0.33	-	-	0.43	-	0.40	-
0336 - 50	-	3.45	-	-	-	-	-	-	-	6.48	-	-	-	-	27.1
Lead															
0212 - 20	0.078	0.076	0.048	0.032	0.050	-	-	-	0.087	-	-	-	0.217	-	-
0212 - 24	-	-	0.034	0.016	-	-	-	-	0.044	-	-	-	0.029	-	-
0212 - 36	-	-	0.044	0.043	-	0.069	-	-	-	-	-	-	-	-	-
0212 - 46	-	-	0.028	0.019	-	0.029	-	-	0.076	-	-	-	-	-	-
0212 - 49	0.077	0.042	0.027	0.020	0.050	-	0.047	0.090	0.065	0.075	0.040	-	-	0.034	-
0350 - 50	-	-	0.047	0.008	-	-	-	-	0.033	-	-	0.009	-	0.007	-
0336 - 50	-	0.009	-	-	-	-	-	-	-	0.034	-	-	-	-	0.005

Table 4.5 Metal concentrations (mg l^{-1}) in 'total' water from Bleaberry Gill (0212) (5 sites), 'Mossy Thorn Gill' (0350) and 'Moor Intake Gill' (0336), collected between 27.01.82 and 05.08.82. Fig. 3.2 and Table 3.1 give stream and reach locations

Results from 0212 - 20 and -49 are shown in more detail in Table 4.6. Mean values are not given for the other reaches with smaller sample sizes.

stream reach	n	Ca		Zn		Ba		Pb	
		$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
0212 - 20	7	16.0	5.2	0.149	0.055	1.86	0.49	0.084	0.062
0212 - 49	11	13.3	5.7	0.084	0.056	0.78	0.14	0.052	0.022

Table 4.6 Mean aqueous metal concentrations ($\bar{x}$) (mg l^{-1}) with standard deviation (S.D.) for two reaches on Bleaberry Gill (0212), sampled between 27.01.82 and 04.08.82

4.32 <u>Effect of rainfall and flow</u>

Rainfall data are given in Table 3.4 and Fig. 3.5 and a discussion of the state of flow of the streams during the study period is given in Section 3.61. The 'total' aqueous metal concentrations were related, to some extent, to the state of flow of the streams and the rainfall.

A comparison of Table 3.4 and Table 4.5 shows some general trends.

i) The concentration of calcium was much higher during the dry months of April and May and relatively low after heavy rain in June.

ii) Concentrations of zinc and lead tended to be lower during dry periods and to increase after rain. Zinc showed this to a greater extent

iii) The aqueous barium concentration appeared not to be related to flow (or rainfall)

iv) It will be noted that some reaches did not fit into this pattern, e.g. 0212 - 20 had a very high aqueous lead concentration and relatively high zinc, on 03.08.82, even though the flow was low on this date

4.33 <u>Relationships between metals at one reach on Bleaberry Gill (0212-49)</u>

The relationships between pairs of elements at 0212 - 49 are shown in Fig. 4.2a-f.

Comments

i) Zinc and lead were negatively correlated with calcium ($P < 0.01$)

ii) Zinc was positively correlated with lead ($P < 0.001$)

iii) There was no significant correlation between barium and calcium, zinc or lead

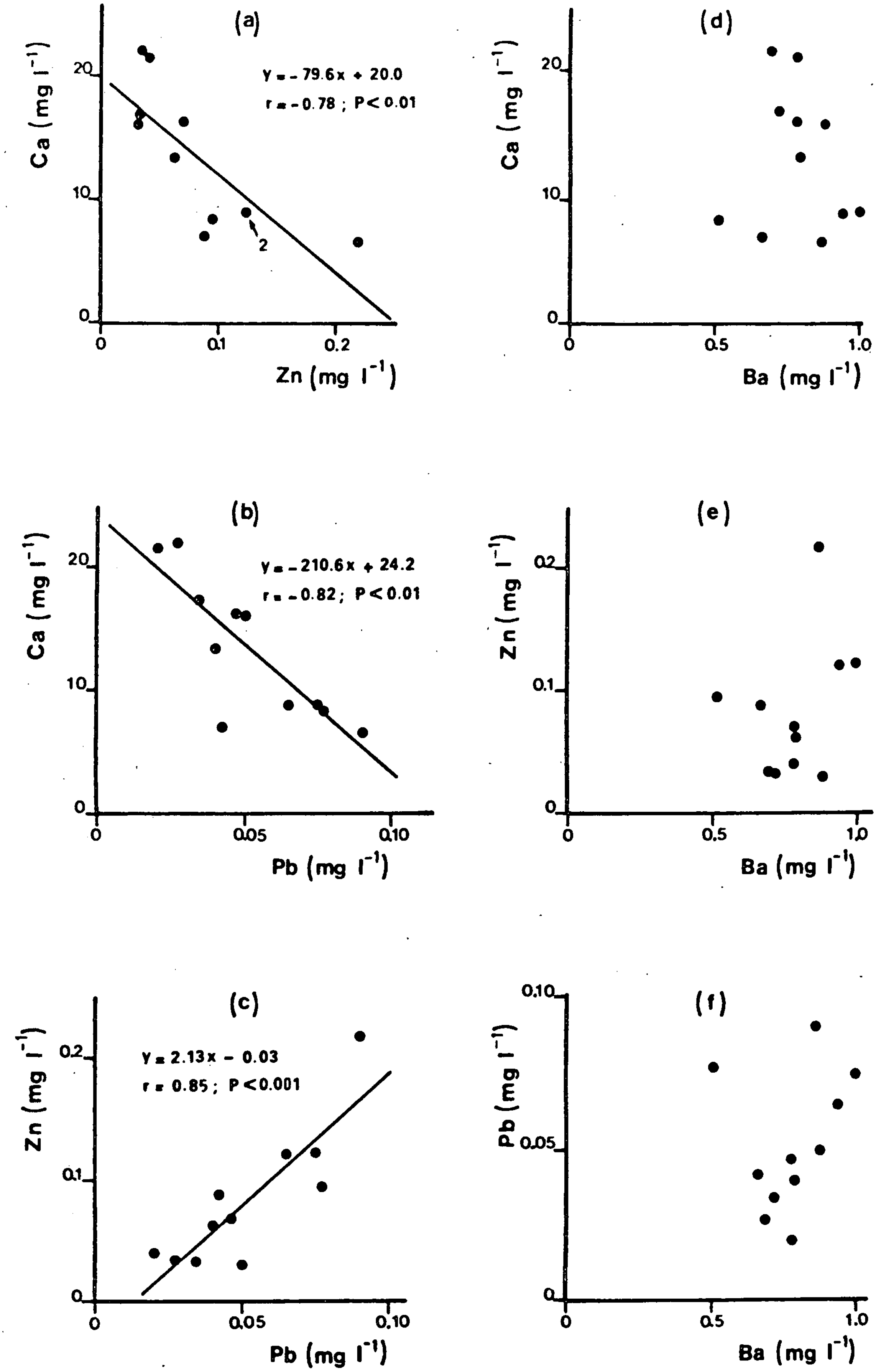


Fig. 4.2a-f Scattergrams showing the relationships between the concentrations of metals in 11 'total' water samples from one reach on Bleaberry Gill (0212 - 49)

(a) Ca against Zn
(b) Ca against Pb
(c) Zn against Pb
(d) Ca against Ba
(e) Zn against Ba
(f) Pb against Ba

CHAPTER 5

RELATIONSHIP BETWEEN METAL CONCENTRATIONS IN *BAETIS* sp(p). AND WATER

A study was planned on one group of animals present in Bleaberry Gill (O212), to compare the concentrations of metal in the group chosen with those in the water. (It had been hoped to analyse the sediments at each reach, but this was not possible in the time available.)

It was necessary to choose a group which both occurred over a wide range of metal concentrations and was generally widespread, since a study on a very localized species would have limited application elsewhere. *Baetis* sp(p). were chosen since they occurred from the top to the bottom of Bleaberry Gill and, as individuals, were of reasonable size for ease of collection. (The Plecoptera were abundant at the time of the animal survey of Bleaberry Gill, 10.05.82, but, such was their life cycles, it could not be guaranteed that they would persist as aquatic forms throughout the summer.)

5.1 COLLECTION AND ANALYSIS OF SAMPLES

Samples of *Baetis* sp(p). and water ('total' and 'filtrable') were collected, as described above (Sections 2.41, 2.31), from sixteen sites including seven on Bleaberry Gill (Section 3.22). Several sites were sampled on more than one occasion. Sites were chosen to give a wide range of metal concentrations, with particular regard to barium.

These samples were analysed for four metals, Ca, Zn, Ba and Pb (Sections 2.23, 2.42).

5.2 RESULTS

Tables 5.1 and 5.2 give the data obtained from this study. Comments are made in Sections 5.21 and 5.22. The relationships between the aqueous metal concentration and metal in *Baetis* sp(p). are discussed in three further sections; one taking an overall view of the results from all sites studied and the other two looking in detail at specific data from Bleaberry Gill.

5.21 Metal concentration in *Baetis* sp(p).

Table 5.1 gives the metal composition of *Baetis* sp(p). collected from sixteen sites, fifteen in the study area (Fig. 3.3) and one on the River Wear near Durham.

Comments

i) At all reaches on Bleaberry Gill the metal concentrations in the animals followed the pattern Zn > Ba > Pb

ii) There was no pattern as above (i) at the other sites, but, except for one occasion (28.06.82 at 0336-50, lead concentrations were the lowest

iii) Coefficients of variation (%) are given where possible. They vary considerably over the range of sites sampled

	Ca	Zn	Ba	Pb
$\bar{x}$ CV	17.5	13.8	25.4	28.6
range	7.9 - 35.6	5.3 - 28.7	18.0 - 36.4	8.8 - 45.6

(Values for barium and lead do not include streams 0371 and 0372 where, due to very low metal concentrations in the digest solutions, which were very near or below the detection limit, the coefficients of variation are probably not representative of the true situation.)

iv) *Baetis* sp(p). were less abundant than expected at 0212-20 and -24.

stream		reach	date	n	Ca $\bar{x}$	Ca ±95%CL	Ca CV(%)	Zn $\bar{x}$	Zn ±95%CL	Zn CV(%)	Ba $\bar{x}$	Ba ±95%CL	Ba CV(%)	Pb $\bar{x}$	Pb ±95%CL	Pb CV(%)
0212	-	20	240682	1	6670	-	--	2330	-	-	1920	-	-	250	-	-
0212	-	24	240682	5	1370	241	14.1	1870	290	12.5	1040	468	36.4	476	202	34.2
0212	-	24	030882	1	1520	-	-	1920	-	-	490	-	-	237	-	-
0212	-	36	010682	1	1300	-	-	2490	-	-	371	-	-	127	-	-
0212	-	45	240682	5	2120	365	13.9	3280	253	6.2	834	218	21.0	196	72	29.6
0212	-	46	010682	1	2470	-	-	3220	-	-	494	-	-	97	-	-
0212	-	46	240682	5	2020	337	13.4	2980	382	10.3	744	226	24.4	215	31	11.6
0212	-	49	230682	5	2100	303	11.6	3660	239	5.3	1260	301	19.2	249	77	25.0
0212	-	49	240682	5	2160	479	17.8	3270	562	13.8	785	218	22.2	151	74	39.4
0212	-	49	040782	5	3390	706	16.8	2890	234	6.5	969	289	24.0	286	31	8.8
0212	-	49	040882	5	2560	446	14.0	3780	1020	21.8	338	130	30.9	140	30	17.5
0212	-	99	230682	5	2460	1090	35.6	4740	707	12.0	703	243	27.8	270	153	45.6
0323	-	99	240682	1	5830	-	-	3450	-	-	9740	-	-	821	-	-
0350	-	50	240682	5	3030	864	22.9	1990	709	28.7	3600	908	20.4	370	181	39.4
0350	-	50	310782	5	3380	398	9.5	2490	384	12.4	4540	1020	18.0	717	282	31.7
0350	-	50	040882	5	3640	460	7.9	2030	722	22.4	5740	3240	35.5	779	390	31.4
0336	-	50	280682	1	3120	-	-	6580	-	-	5640	-	-	689	-	-
0336	-	50	050882	1	3790	-	-	7890	-	-	6640	-	-	711	-	-
0336	-	85	050882	1	4860	-	-	6670	-	-	8160	-	-	1300	-	-
0371	-	98	020882	5	2100	596	22.9	246	28	9.2	<155	-	-	2.6	2.6	79.8
0372	-	60	020882	5	3200	1100	27.7	433	102	18.9	<307	-	-	7.5	4.8	51.6
0328	-	10	070882	1	4800	-	-	3490	-	-	5860	-	-	2125	-	-
0374	-	85	020882	1	7420	-	-	10600	-	-	6200	-	-	1583	-	-
0008	-	65	040682	1	2380	-	-	936	-	-	48	-	-	83	-	-

Table 5.1 Metals (µg g^{-1} dry wt) in *Baetis* sp(p). collected from 16 sites, 01.06.82 - 07.08.82. Table 3.1 and Figure 3.3 give stream and reach locations. Sample size (n), mean ± 95% confidence limits of the mean ($\bar{x}$ ± 95%CL) and coefficient of variation (CV) are given

stream	reach	date	Ca		Zn		Ba		Pb	
			T	F	T	F	T	F	T	F
0212 -	20	24.06.82	14.8	14.4	0.26	0.26	2.30	2.30	0.087	0.067
0212 -	24	24.06.82	8.1	8.0	0.202	0.196	1.37	1.48	0.044	0.038
0212 -	24	03.08.82	5.23	(6.37)	0.050	0.047	0.62	(0.85)	0.029	0.010
0212 -	36	01.06.82	8.9	8.9	0.047	0.026	0.77	0.76	0.069	0.013
0212 -	45	24.06.82	6.8	7.0	0.152	0.154	0.92	0.89	0.077	0.069
0212 -	46	01.06.82	17.4	17.4	0.027	0.028	0.59	0.59	0.029	0.015
0212 -	46	24.06.82	9.0	8.9	0.166	0.160	1.00	0.97	0.076	0.057
0212 -	49	23.06.82	6.6	7.1	0.219	0.200	0.87	0.80	0.090	0.074
0212 -	49	24.06.82	8.8	8.5	0.122	0.130	0.94	0.93	0.065	0.044
0212 -	49	04.07.82	13.5	-	0.063	-	0.79	-	0.040	-
0212 -	49	04.08.82	17.3	17.5	0.034	0.016	0.72	(0.93)	0.034	0.021
0212 -	99	23.06.82	9.2	8.7	0.23	0.21	0.87	0.75	0.099	0.068
0323 -	99	24.06.82	10.7	10.4	0.29	0.27	3.08	3.11	0.109	0.078
0350 -	50	24.06.82	29.1	29.0	0.032	0.028	0.33	0.34	0.033	0.012
0350 -	50	31.07.82	32.3	32.1	0.015	0.009	0.43	0.41	0.009	0.004
0350 -	50	04.08.82	32.5	32.3	0.011	0.012	0.40	0.38	0.007	0.005
0336 -	50	28.06.82	42.2	-	0.27	-	6.48	-	0.034	-
0336 -	50	05.08.82	72.7	-	0.103	-	27.1	-	0.005	-
0336 -	85	05.08.82	69.6	68.5	0.070	0.019	18.4	15.3	0.044	0.004
0371 -	98	02.08.82	15.8	15.9	0.005	<0.005	0.14	(0.19)	0.004	<0.003
0372 -	60	02.08.82	35.3	35.0	0.006	<0.005	0.24	0.24	<0.003	<0.003
0328 -	10	07.08.82	53.2	52.8	0.098	0.095	0.34	(0.79)	0.029	0.015
0374 -	85	02.08.82	41.2	41.1	0.129	0.113	1.82	1.61	0.196	0.194
0008 -	65	04.06.82	104.6	104.8	0.046	0.033	0.32	0.32	0.006	0.005

Table 5.2 Metals (mg l^{-1}) in water collected from 16 sites, 01.06.82 - 07.08.82. T = 'total' F = 'filtrable' (values in brackets indicate instances where contamination has probably occurred, Section 5.22). Table 3.1 and Figure 3.3 give stream and reach locations

5.22 Water Chemistry

Table 5.2 gives the concentration of Ca, Zn, Ba and Pb in 'total' and 'filtrable' water from each reach at the time of sampling for *Baetis* sp(p).

Comments

i) There was little difference between 'total' and 'filtrable' calcium and barium concentrations. Zinc also showed little difference except at some sites (0212-49, 04.08.82 and 0336-85, 05.08.82).

ii) The 'total' and 'filtrable' lead concentrations did differ at each reach sampled

iii) The following ranges of 'total' aqueous metal concentrations were sampled:

	Ca	Zn	Ba	Pb
range (mg l^{-1})	5.23 - 104.6	0.005 - 0.29	0.14 - 27.1	<0.003 - 0.196

iv) Several sites were sampled on more than one occasion. Large variation occurred in all four metals with time. This was analysed in more detail (Section 4.3)

v) Some values in Figure 5.2 are in brackets to indicate that contamination has probably occurred. This was during one week at the beginning of August when samples were being regularly taken from 'Moor Intake Gill' (0336) which, during that period, had a very high aqueous barium concentration

5.23 Relationship between metals in *Baetis* sp(p). and water

Figures 5.1a-d show the relationships between the concentration of Ca, Zn, Ba and Pb in *Baetis* sp(p). and 'total' water samples collected at the same time. Comparisons between the metal concentration in *Baetis* sp(p). and 'filtrable' water samples are not presented here. Data are given in Tables 5.1, 5.2.

Comments

i) The concentration of calcium in *Baetis* sp(p). tended to increase with an increase in aqueous metal concentration; this was not a significant relationship

ii) There was no relationship between zinc and lead tissue concentrations and aqueous metal concentrations. The concentration of zinc in animal samples from 'Moor Intake Gill' (0336) and Old Gang Beck (0374) were substantially higher than those from Bleaberry Gill (0212), Moulds Old Level (0328) and 'Fourth Whim Sike' (0323), the latter having comparable aqueous zinc concentrations. Lead tissue concentrations tended to be lower at reaches on Bleaberry Gill than in animal samples taken from other streams with comparable aqueous lead

iii) The concentration of barium in *Baetis* sp(p). appeared to depend on the stream from which the animals came. There was a significant positive linear relationship between tissue and aqueous metal concentrations ($P < 0.01$) (using data from all streams). Bleaberry Gill alone also showed such a relationship ($P < 0.001$, Fig. 5.1c)

iv) Annaside Beck (0371) and Roe Beck (0372), streams not affected by mining activity and with low aqueous zinc, barium and lead, contained animals with low concentrations of these metals

v) 'Mossy Thorn Gill' (0350), with relatively low aqueous zinc, barium and lead, contained animals with high concentrations of these metals, particularly barium. Moulds Old Level (0328) similarly contained animals with very high barium concentrations although the aqueous barium was low

Figure 5.1a-d Relationship between metals in *Baetis* sp(p). and 'total' water. Aqueous metal concentrations in mg l^{-1}, tissue metal concentrations in $\mu g\ g^{-1}$ dry wt.

Vertical bars represent 95% confidence limits of the mean

Key to streams:

●	Bleaberry Gill	0212
○	'Fourth Whim Sike'	0323
■	'Mossy Thorn Gill'	0350
□	'Moor Intake Gill'	0336
◆	Annaside Beck	0371
◇	Roe Beck	0372
▲	Moulds Old Level	0328
△	Old Gang Beck	0374
✚	R. Wear	0008

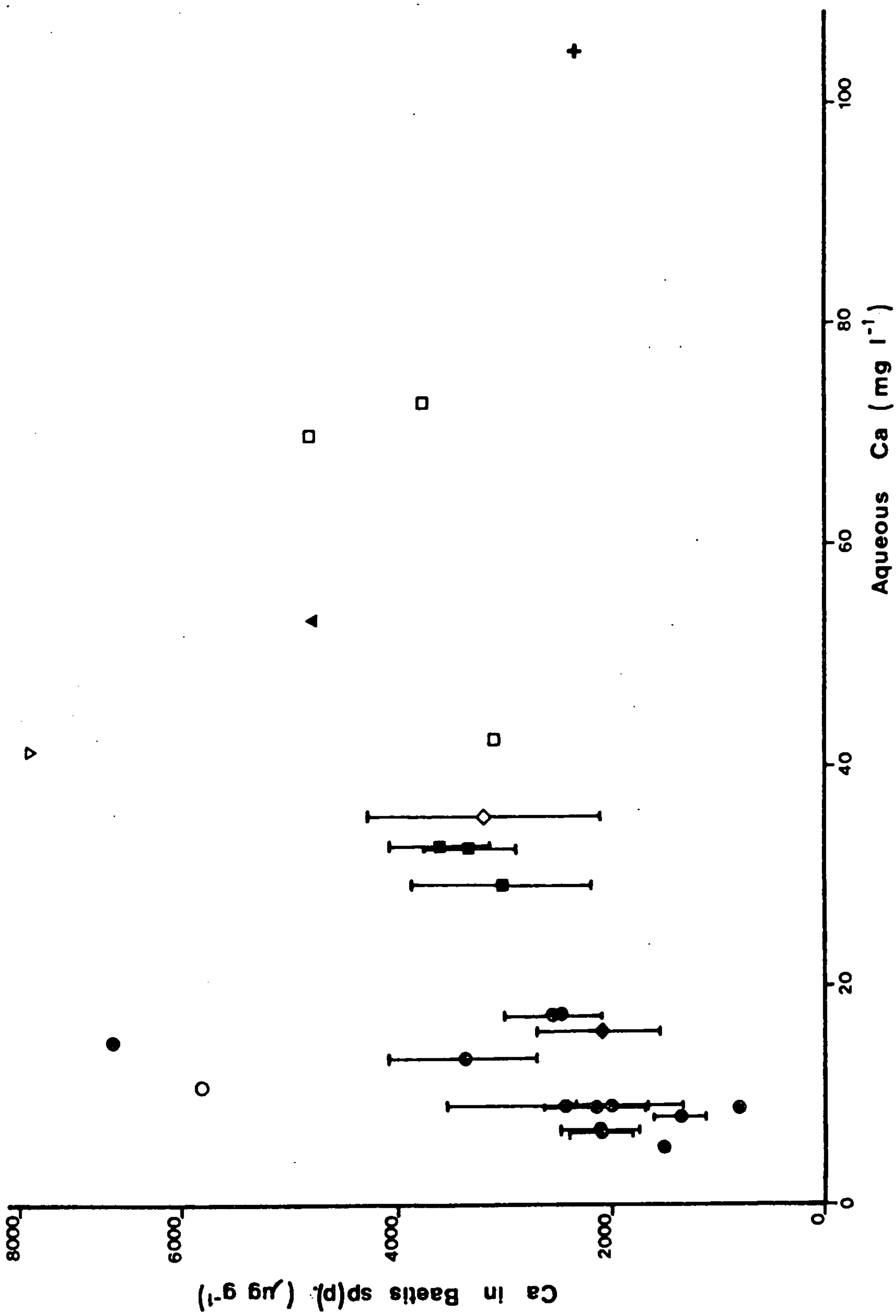


Figure 5.1a Calcium

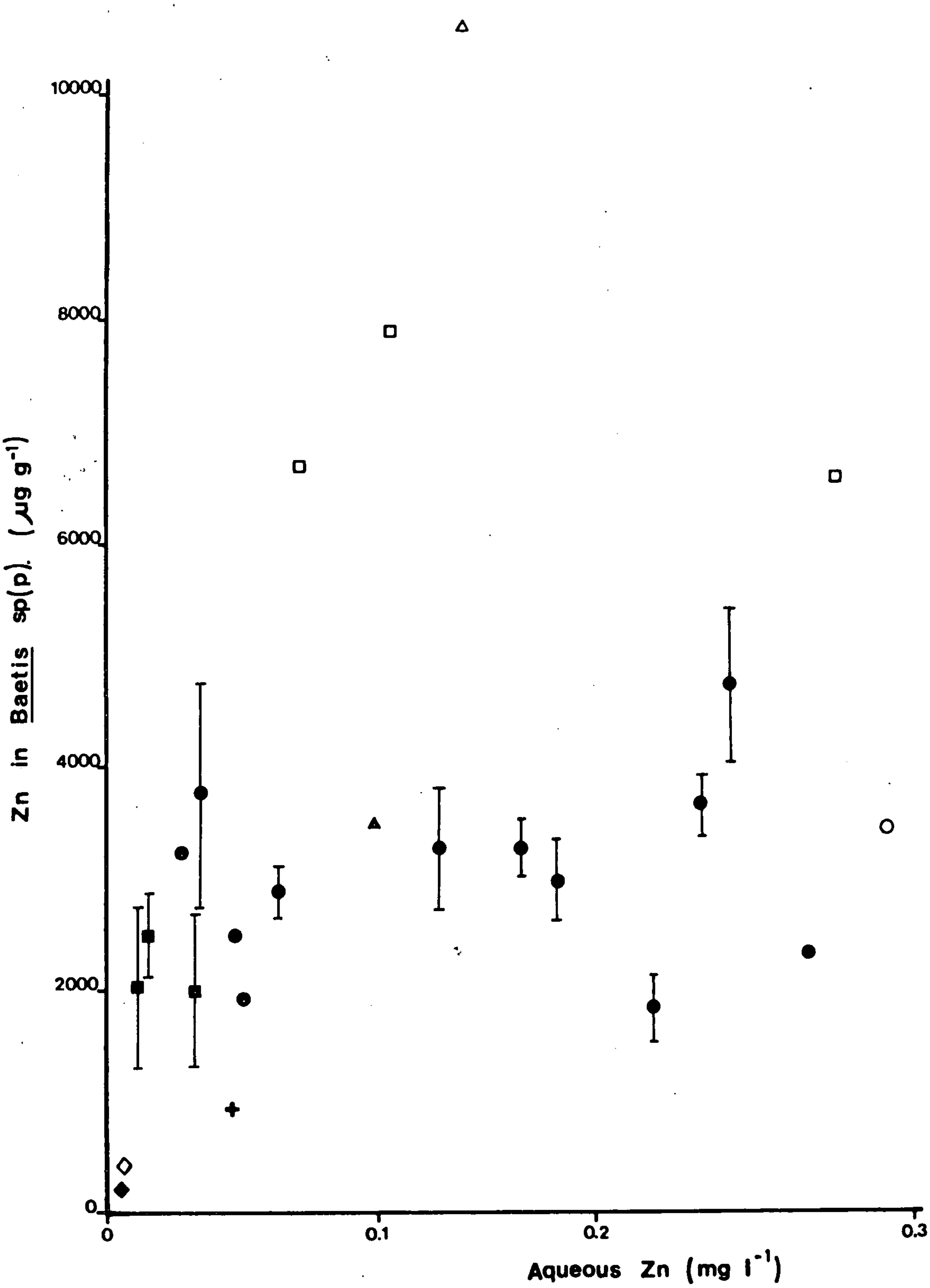


Figure 5.1b Zinc

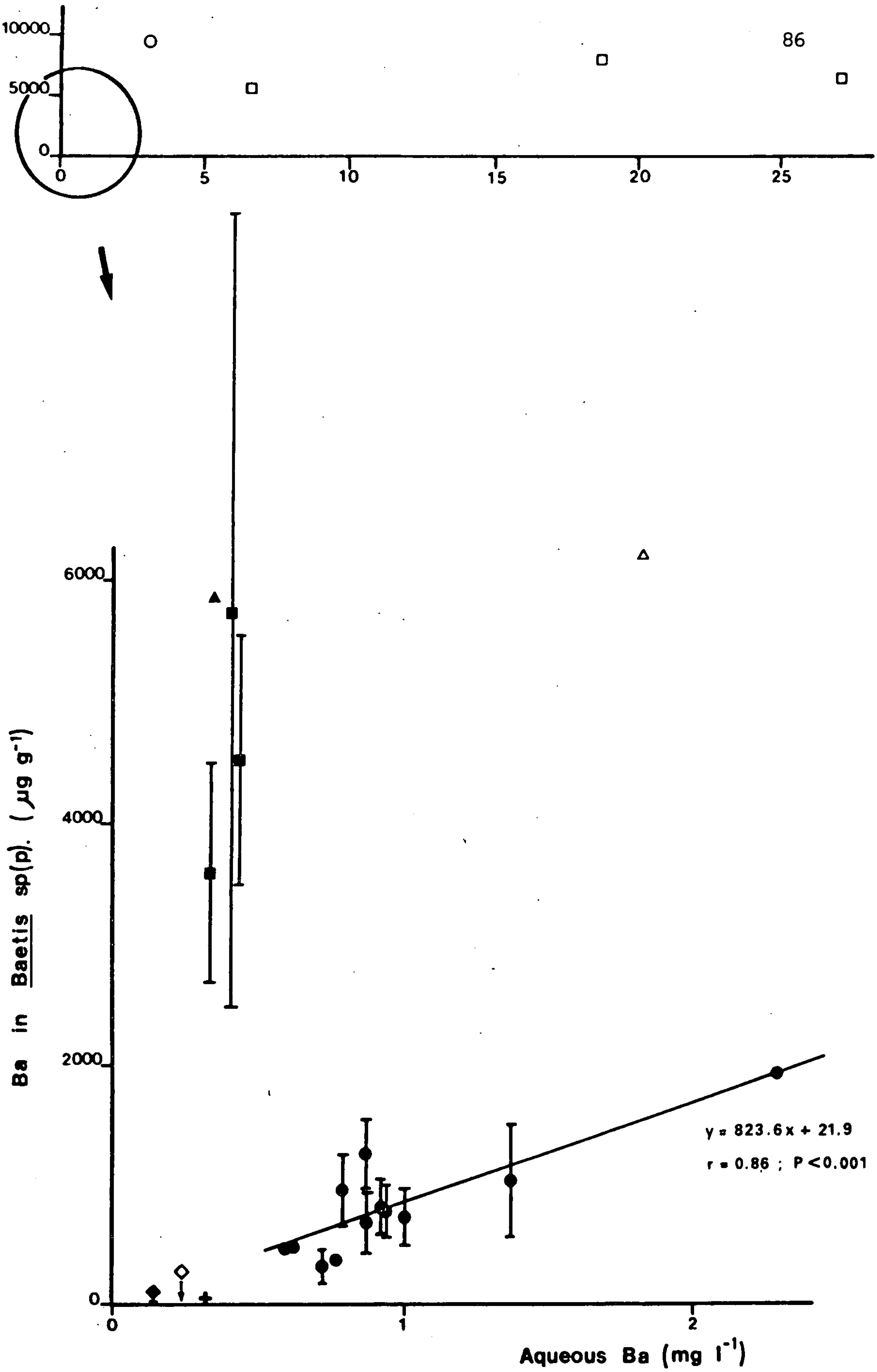


Figure 5.1c Barium

Correlation between barium in *Baetis* sp(p). and 'total' water, using data from Bleaberry Gill

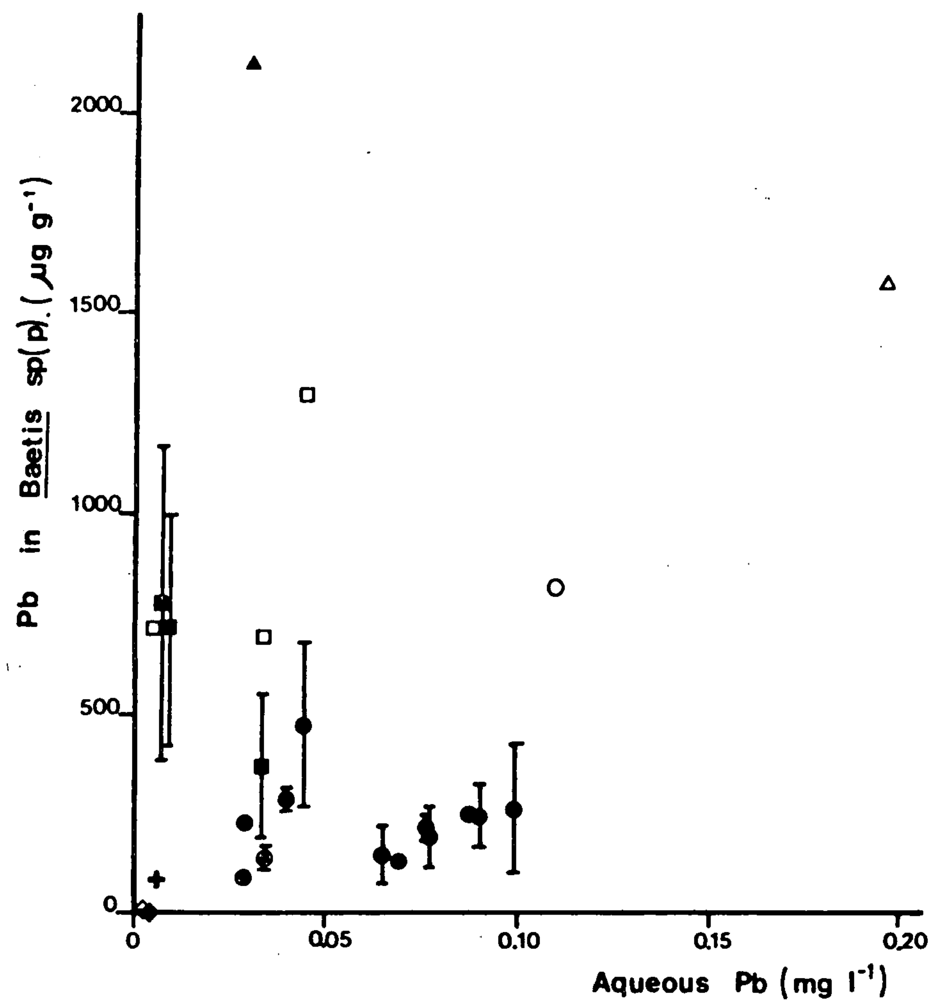


Figure 5.1d   Lead

5.24 Bleaberry Gill profile

Five reaches on Bleaberry Gill (0212) were sampled for *Baetis* sp(p). on 24.06.82. Results are given in Tables 5.1, 5.2 (n = 5 for all reaches except -20 where only one sample could be taken due to shortage of animals). Comparison was made between the metal concentration in the animals and 'total' water at each reach (Fig. 5.2a-d). Analysis of variance and *t*-tests comparing each reach were carried out.

Comments

i) There was no significant difference between the metal concentrations in *Baetis* sp(p). at reaches 45, 46 and 49

ii) Calcium and zinc concentrations in animals from reach 24 were significantly lower than the other reaches ($P < 0.01$, 0.001 respectively), although there was no fall in the aqueous metal concentrations at this reach. The lead concentration in animals at this reach was significantly higher although it had a substantially lower aqueous lead concentration

iii) Animals from reach 20 had substantially higher calcium and barium concentrations than animals from the four other reaches

iv) There appears to be a trend of decreasing barium concentration in *Baetis* sp(p). with decreasing aqueous barium. However, since there was no significant difference between tissue barium at reaches 24, 45, 46 and 49, this trend is based on one sample from reach 20. It is obvious that replicates should have been taken at this site. The coefficient of variation, of barium concentrations, was higher in animals from reach 24 than reaches 45, 46 and 49

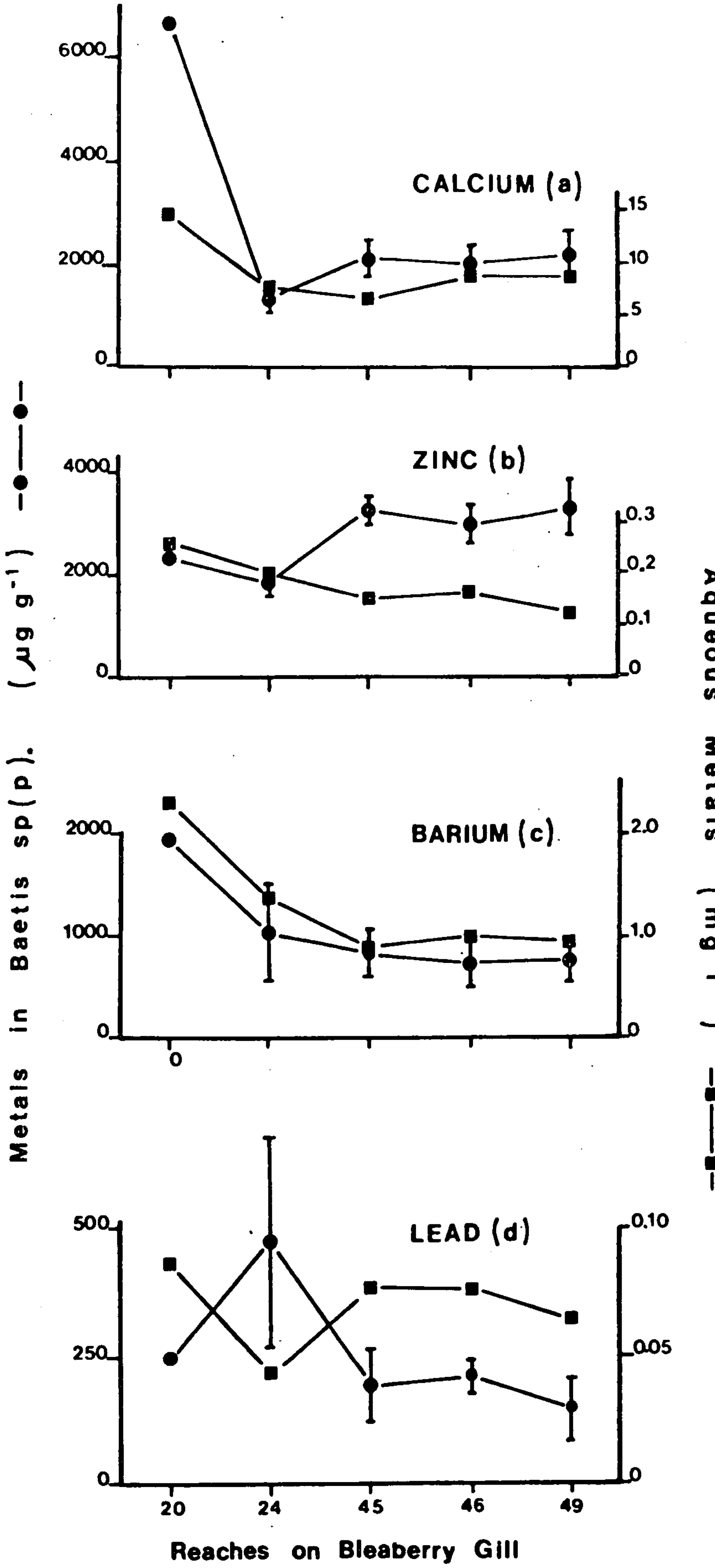


Figure 5.2a-d Metals in *Baetis* sp(p). (µg g^{-1} dry wt) compared with 'total' aqueous metal (mg l^{-1}) at five reaches on Bleaberry Gill, 24.06.82

Vertical bars represent 95% confidence limits of the mean

5.25 Metals in *Baetis* sp(p). at one reach on Bleaberry Gill (0212-49)

This study was carried out to determine variation with time in the metal concentration and content of *Baetis* sp(p). at one site and to compare this variation with the variation in aqueous metal concentrations.

Reach 49 on Bleaberry Gill (0212) was sampled for *Baetis* sp(p). on four occasions in late June, July and August. Metal concentration and mean metal content per individual organism are given in Table 5.3, aqueous metal concentrations are shown in Table 5.2.

Analysis of variance and *t*-tests were carried out on the results.

It was noticed that individuals collected in August were larger than in the two previous months (Table 5.3). The mean individual dry weight for August was found to be significantly higher ($P < 0.001$). It is probable that these animals were of a later instar, moult having taken place between 04.07.82 and 04.08.82.

5.251 Metal concentration

There was no obvious relationship between the concentration of metals in *Baetis* sp(p). and the aqueous metal concentration at the time of sampling. The barium concentration in the animals showed a very significant decrease in August when the aqueous barium was only slightly lower than on previous occasions ($P < 0.001$ with 23.06.82 and 04.07.82, $P < 0.01$ with 24.06.82).

5.252 Metal content

There was no obvious relationship between the zinc, barium and lead content of individuals of *Baetis* sp(p). and the aqueous metal concentration at the time of sampling. Animals collected in August

Date	Dry weight/ individual (mg) $\bar{x}$	S.D.		Ca $\bar{x}$	Ca ±95%CL	Ca CV(%)	Zn $\bar{x}$	Zn ±95%CL	Zn CV(%)	Ba $\bar{x}$	Ba ±95%CL	Ba CV(%)	Pb $\bar{x}$	Pb ±95%CL	Pb CV(%)
23.06.82	0.96	0.10 n=5	1	2100	303	11.6	3660	239	5.3	1260	301	19.2	249	77	25.0
			2	2.01	0.25	10.0	3.50	0.25	5.7	1.19	0.18	11.8	0.235	0.059	20.0
24.06.82	1.13	0.27 n=5	1	2160	479	17.8	3270	562	13.8	785	218	22.2	151	74	39.4
			2	2.40	0.48	16.2	3.63	0.76	16.8	0.87	0.23	21.8	0.163	0.064	31.3
04.07.82	1.01	0.06 n=5	1	3390	706	16.8	2890	234	6.5	969	289	24.0	286	31	8.8
			2	3.44	0.85	19.8	2.92	0.23	6.2	0.98	0.30	24.5	0.290	0.039	10.7
04.08.82	1.72	0.21 n=5	1	2560	446	14.0	3780	1020	21.8	338	130	30.9	140	30	17.5
			2	4.43	1.26	22.8	6.53	2.11	26.0	0.59	0.33	44.1	0.244	0.086	28.3

Table 5.3 Metal concentration and content in *Baetis* sp(p). at one reach on Bleaberry Gill (0212-49) sampled on four occasions

1 metal concentration (μg g^{-1} dry wt) (from Table 5.1)

2 metal content per individual (μg)

contained significantly more zinc/individual than the other three samples ($P < 0.001$), and significantly less barium/individual than 23.06.82 ($P < 0.001$) and 04.07.82 ($P < 0.01$). The calcium content of the animals increased over the sampling period, 04.08.82 samples being significantly higher than 23.06.82, 24.06.82 ($P < 0.001$) and 04.07.82 ($P < 0.05$).

Sections 5.251 and 5.252 show a change in the metal concentration and content with the stage of life cycle. Animals collected in August were of a later instar. Barium concentration and content decreased in this stage. It is possible that, at moult, barium is lost from the body, perhaps in the exoskeleton. Zinc however may be retained during moult, after which uptake continues to result in a higher zinc content per individual.

CHAPTER 6

METAL ACCUMULATION BY *GAMMARUS PULEX* (L.)

It was decided to study metal accumulation by one group of animals in more detail. *Gammarus pulex*, the freshwater shrimp, was chosen for this since it occurred in very large numbers in 'Mossy Thorn Gill' (0350). This allowed for sufficient animals to be removed, for both laboratory and field investigations, without causing drastic damage to the population. Further, although *G. pulex* was not a common invertebrate of Bleaberry Gill (Section 4.23), it is a widespread organism of upland streams and lowland rivers.

Two investigations were carried out; a laboratory study on barium uptake and a field study involving the transplant of animals from a barium poor stream to a barium rich stream. As the effect of the presence of gut contents on the measurements of metal concentrations was not known, a preliminary study was carried out (Section 6.1).

6.1 INFLUENCE OF THE PRESENCE OF GUT CONTENTS ON MEASUREMENTS OF METAL CONCENTRATIONS IN *GAMMARUS PULEX*

The concentration of metals in animals that feed on metal-rich food (plant and animal material and detritus) depends to some extent on the quantity of food in their guts at the time of sampling. This experiment was set up to determine:

a) the concentration of metals in *G. pulex* with and without gut contents

b) whether the gut contents formed a significant part of the dry weight of each sample.

Gammarus pulex individuals were collected from 'Mossy Thorn Gill' (0350-50). Five samples were taken immediately and the remaining animals were kept without food for >18 hours to allow them to clear their guts (Section 2.6). After this time samples were taken and no faecal pellets were seen in the animals or collection vials. A wide range of gut passage times have been reported for *Gammarus* spp. ranging from 2 hours or less (Marchant and Hynes, 1981; Bärlocher and Kendrick, 1975), up to 12 hours (Monk, 1977; Sutcliffe *et al.*, 1981) and 2 - 48 hours (Willoughby and Earnshaw, in press). The time needed appears to depend on the type of food and the temperature. This experiment was also carried out using *Baetis* sp(p). but all but six specimens died. No *G. pulex* died during the experiment.

6.11 Metal composition

		Ca			Zn			Ba			Pb		
	n	$\bar{x}$	±95%CL	CV(%)	$\bar{x}$	±95%CL	CV(%)	$\bar{x}$	±95%CL	CV(%)	$\bar{x}$	±95%CL	CV(%)
A (with)	5	135000	13600	8.1	171	30	13.9	4500	1040	18.6	494	134	30.4
B (without)	5	146000	11400	6.3	115	10	7.0	2110	195	7.4	198	18	7.4

Table 6.1 Metal composition of *G. pulex* with and without gut contents ($\mu g\ g^{-1}$ dry wt)

Sample size (n), mean ± 95% confidence limits of the mean ($\bar{x}$ ± 95%CL) and coefficient of variation (CV) are given

Table 6.1 (above) shows the results for *G. pulex* with gut contents (A) and without gut contents (B). *t*-tests for significant difference showed that A and B were not significantly different for Ca ($P > 0.10$), but were significantly different for Zn ($P < 0.01$), Ba ($P < 0.001$) and Pb ($P < 0.01$).

Comments

i) Significantly higher levels of zinc, barium and lead, but not calcium, were measured in animals with gut contents (A) than those without (B).

ii) The gut contents provided a substantial proportion of the metals measured in *G. pulex*; about 33% of the zinc, 53% of the barium and 60% of the lead

iii) The coefficient of variation was consistently less for zinc, barium and lead in animals without gut contents. The approximate proportion of the coefficient of variation due to variation in metals in the gut contents was zinc 50% barium 60% lead 76%

6.12 Effect of gut contents on dry weight

Table 6.2 shows the dry weight of each animal sample, with and without gut contents.

	sample	dry wt(mg)	$\bar{x} \pm$ 95% CL			dry wt(mg)	$\bar{x} \pm$ 95% CL	
(A)	1	30.0	29.3	2.0	(B)	33.1	30.0	2.9
	2	27.4				30.4		
	3	28.6				31.0		
	4	31.7				28.3		
	5	28.6				27.1		

Table 6.2 Dry weight (mg) of five *G. pulex* samples with (A) and without (B) gut contents. Mean values ± 95% confidence limit of the mean ($\bar{x}$ ± 95% CL) are given

Comments

i) There was no significant decrease in the dry weight of *G. pulex* samples without gut content ($P > 0.10$) (*t*-test)

ii) A high proportion of the metal in the animals was provided by a very low dry weight of gut content material

6.2 BARIUM UPTAKE BY *GAMMARUS PULEX* (L.) - laboratory study

It was not known if, or to what extent, *G. pulex* may take up barium. This laboratory study was therefore carried out, before a subsequent field study (Section 6.3), to determine if it does take up barium and, if so, to give some indication of the length of time needed to demonstrate uptake and the sampling intervals required.

The gut contents of *G. pulex* affect the concentration of metal measured and contribute to a large proportion of the variation between samples (Section 6.1). It was decided to study uptake from solution only, therefore eliminating some of the problems caused by variation in gut contents. Any increase in barium concentration would be due to adsorption from the water onto the cuticle, uptake across the respiratory surfaces and through the gut wall.

G. pulex was transferred from 'Mossy Thorn Gill' (0350-50) (0.33 - 0.43 mg l^{-1} Ba; Table 4.4) to tank A (- Ba) and tank B (+ Ba) as described in Section 2.7. Water and animal samples were taken over a 72-hour period (Section 2.731) and were analysed for barium (Section 2.75). Animals left at the end of the 72-hour sampling period were transferred back into 'filtrable' 'Mossy Thorn Gill' water and samples were taken over the next 11 days (Section 2.74). Although at this stage of the experiment it was not known if there had been uptake of barium, this was assumed and the investigation was continued to see if there was any subsequent loss.

6.21 Influence of elevated aqueous barium on barium in *G. pulex*

Barium concentrations in *G. pulex* over the three day exposure to high aqueous barium (6mg l^{-1}) are given in Table 6.3 and Figure 6.1.

	t (hours)	n	Barium concentration		
			$\bar{x}$	±95%CL	CV(%)
A (- Ba)	0	5	1450	73	4.1
	24	3	1210	289	9.6
	48	3	1330	90	2.7
	72	5	1420	109	6.2
B (+ Ba)	0	5	1450	73	4.1
	1	5	1300	101	6.3
	2	4	1360	138	6.3
	4	5	1370	77	4.5
	8	5	1480	42	2.3
	12	5	1610	274	13.7
	24	5	1480	58	3.2
	36	5	1790	264	11.9
	48	5	2000	298	12.0
	72	5	2080	472	18.3

Table 6.3 Influence of high aqueous barium (6mg l^{-1}) on barium in *G. pulex* (µg g^{-1} dry wt). Sample size (n), mean ± 95% confidence limits of the mean ($\bar{x}$ ± 95%CL) and coefficient of variation (CV) are given

Note: t + 0 (A) is the same as t + 0 (B)

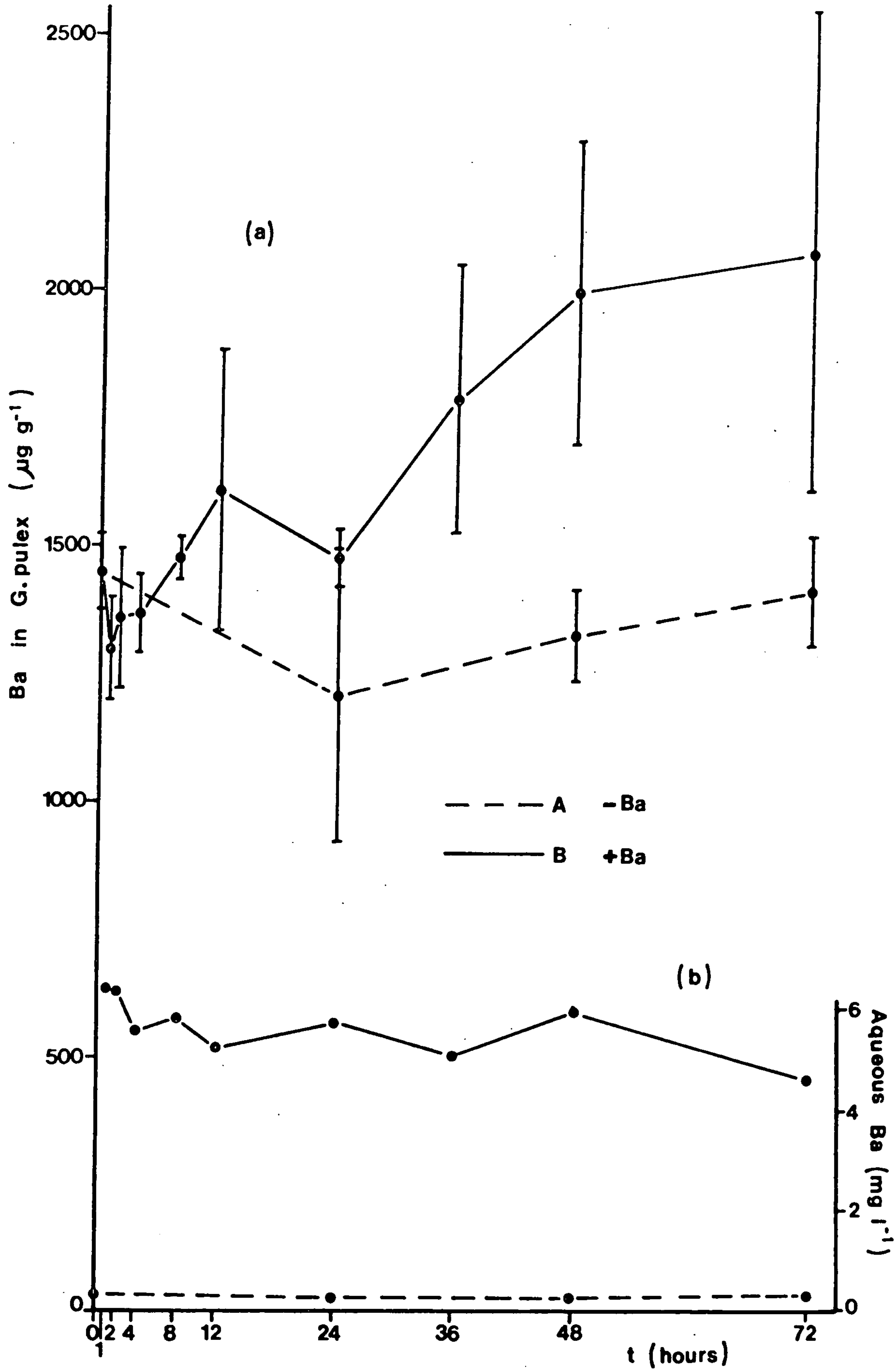


Figure 6.1 Influence of high aqueous barium (6mg l^{-1}) on the barium concentration in *G. pulex*

(a) Barium in *G. pulex* ($\mu g\ g^{-1}$ dry wt)
(b) Aqueous barium (mg l^{-1})

Vertical bars represent 95% confidence limits of the mean

Comments

i) The animals experienced an increase in aqueous barium concentration of about 6mg l^{-1}. The concentration of barium in *Gammarus* increased by 43.4%

ii) The concentration of barium in animals in tank A (- Ba) differed significantly from animals in tank B (- Ba), after 24 hours exposure.

$t + 24$ $P < 0.01$, $t + 48$ $P < 0.001$, $t + 72$ $P < 0.01$ (t-test)

This is discussed in Section 6.211 where further analysis shows at least 36 hours were needed to be certain of a significant increase in the barium concentration

iii) Barium in *Gammarus* appeared to reach an equilibrium concentration at 2 - 3 days

iv) The mean rate of barium uptake over three days was 210µg g^{-1} dry wt day^{-1}

v) The coefficient of variation tended to increase over the sampling period

vi) The aqueous barium concentration in tank A remained steady throughout the sampling period

vii) The aqueous barium concentration in tank B showed a 27% decrease over the sampling period. Since this decrease was not regular, it is possible that the concentration varied substantially throughout the tank

6.211 Statistical analysis

When *t*-tests were carried out pair-wise on barium concentrations in *G. pulex*, at t + 24, t + 48 and t + 72 hours, from A (no added barium) and B (with added barium), animals from B were found to have a significantly higher barium concentration than those from A. However, the barium concentration in animals from B at t + 24 hours did not fit smoothly into the uptake curve (Fig. 6.1) and was found to be not significantly higher than t + O, the starting concentration (Table 6.4).

t (hours)	12	24	36	B(+ Ba)
O	N.S.	N.S.	**	
24	*	**	**	
48	N.S.	**	*	

A(- Ba)

Table 6.4 Comparison between barium concentrations in *G. pulex* exposed to high (B) and low (A) barium after 12, 24 and 36 hours

* $P < 0.05$ (*t*-tests)
** $P < 0.01$

Further analysis (Table 6.4) suggests that 36 hours were needed for *G. pulex* to accumulate barium to a concentration significantly above the starting concentration.

6.22 Influence of low aqueous barium on barium in *G. pulex* after exposure to high aqueous barium

Barium concentrations in *G. pulex* over the 11 days after transfer back to A (- Ba) from B (+ Ba) are given in Table 6.5 and Figure 6.2. One animal and water sample was taken at each sampling time; no confidence limits can be given. Aqueous metal concentrations during the 11 day sampling period are shown in Figure 6.2.

t (hours)	Barium concentration
1	2000
2	2230
5	4345
8	2280
12	2170
24	2340
36	3230
48	5650
72	2190
96	4184
120	2300
144	2410
168	2560
264	2240

Table 6.5 Influence of low aqueous barium on barium in *G. pulex* ($\mu g\ g^{-1}$ dry wt) over 11 days after exposure to high aqueous barium

Comments

i) Barium concentration in *G. pulex* did not return to its initial level during the 11 days of the investigation

ii) Barium concentration in *G. pulex* at t + 5, t + 48 and t + 96 hours was much higher than at other sampling times, possibly due to the presence of individuals with exceptionally high barium

iii) Aqueous barium concentration remained steady throughout the investigation

6.23 Mortality

Mortality was recorded throughout the uptake study but not during the loss study. Results for the former are shown below:

t(hours)	0	1	2	4	8	12	24	36	48	72
A - Ba	0	0	0	0	0	0	2	2	0	1
B + Ba	0	0	0	0	0	0	1	1	3	0

Comments

i) There was no difference in the mortality of the animals exposed to high and low barium

ii) No individuals died during the first 12 hours of the investigation

iii) Live *Gammarus* were seen to eat dead or dying individuals. All dead or dying animals and cast skins were removed from the tanks on each sampling occasion

iv) It was not known if individuals had died and been eaten between sampling times

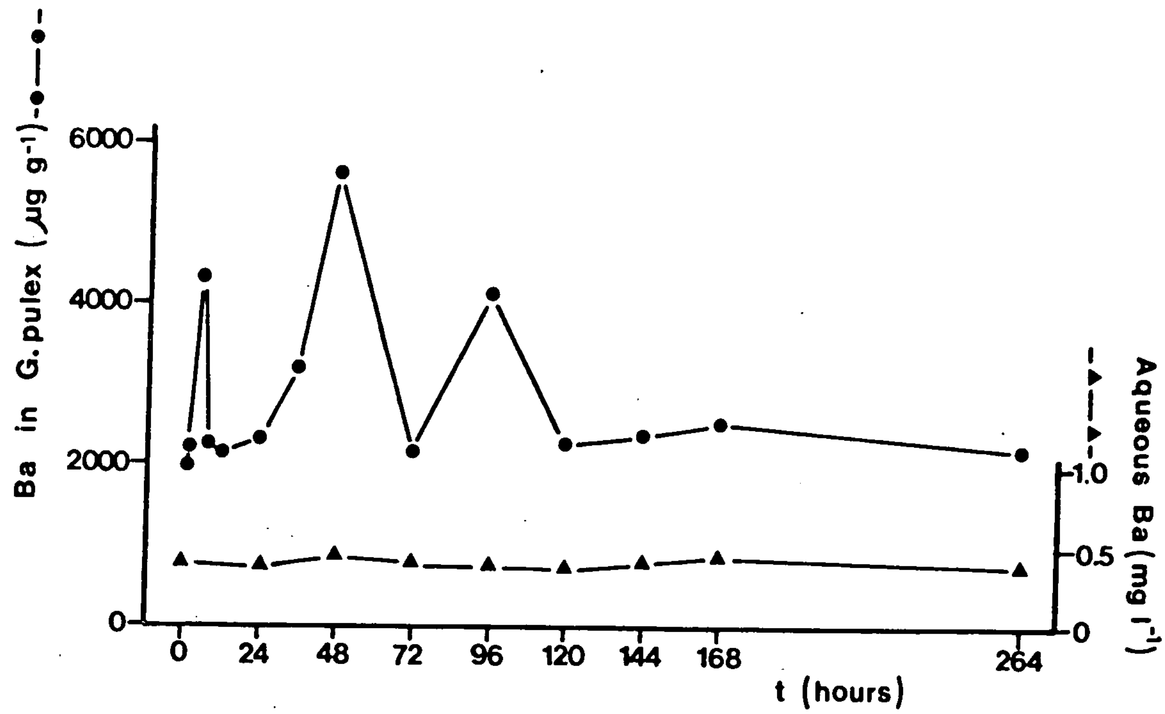


Figure 6.2 Influence of low aqueous barium on barium in *G. pulex* (μg g^{-1} dry wt) over 11 days following exposure to high aqueous barium (6mg l^{-1})

6.3 METAL UPTAKE BY *GAMMARUS PULEX* - field transplant study

It was shown in Section 6.2 that *Gammarus pulex* does take up barium from the water. A transplant experiment was set up to demonstrate barium uptake in a field situation. It was decided to transfer a large number (800) of *G. pulex* from 'Mossy Thorn Gill' (0350), with aqueous barium concentrations of 0.33 - 0.43mg l^{-1} (Table 4.4), to 'Moor Intake Gill' (0336) which had barium concentrations of 3.45mg l^{-1} (16.03.82) and 6.48mg l^{-1} (28.06.82). The latter was similar to that used in the laboratory investigation (Section 6.2). Invertebrate samples taken from 'Moor Intake Gill' showed that the stream did not previously contain *G. pulex*, even though substrate and flow conditions were thought to be suitable.

The laboratory study showed that three days were needed before a 'levelling off' of the barium concentration in the animals occurred. It was decided to run the field study for a longer period and five days was chosen. The sampling intervals were kept the same as in the laboratory study, except the t + 1 sample was omitted to prevent disturbance so soon after the transplant. No measurable increase occurred after this time in the laboratory study.

As in Section 6.2, uptake from solution only was studied. The animals were deprived of all food for > 18 hours before the transplant and were held in a transplant cage (Section 2.81 and Figs. 2.2a,b) to prevent access to food particles. They did, however, feed to a small extent on particles trapped by the mesh of the cage. They were also seen eating dead or dying individuals. All dead and dying animals were removed from the cage at each sampling time; it was not known to what extent metals were taken up from this source.

'Moor Intake Gill' also had higher aqueous calcium and zinc than 'Mossy Thorn Gill'. The animal and water samples were therefore analysed for these metals as well as barium. Aqueous lead concentration, temperature, conductivity and pH were measured to give more information on the stability of 'Moor Intake Gill' during the transplant period.

6.31 Water chemistry of 'Moor Intake Gill' (0336) during field transplant

The aqueous metal concentration ('total' and 'filtrable'), temperature, conductivity and pH are summarized in Table 6.6.

Comments

i) The barium concentration in 'Moor Intake Gill' was much higher than expected; the average value for 'total' water being 27.7mg l^{-1}

ii) Two t + 0 values are given in Table 6.6, 0350-50 t + 0 gives the chemistry of the water in the transplant cage prior to transfer, 0336-40 t + 0 gives the water chemistry of 'Moor Intake Gill' at the start of the experiment

iii) Calcium levels at 0336-40 were about twice that of 0350-50 and remained relatively constant throughout the study period

iv) The 'total' aqueous zinc concentration measured at 0336-40 was an order of magnitude higher than 0350-50

v) Temperature and conductivity were substantially higher at 0336-40 than 0350-50, while pH was slightly higher

stream - reach	t (hours)	date	Temperature (°C)	Conductivity ($\mu S\ cm^{-1}$)	pH	Ca T	Ca F	Zn T	Zn F	Ba T	Ba F	Pb T	Pb F
0350 - 50	0	02.08.82	8.3	282	7.40	33.4	-	0.014	-	0.45	-	0.009	-
0336 - 40	0	,,	15.4	432	7.90	74.9	70.8	0.139	0.115	27.0	26.7	0.007	< 0.003
,,	2	,,	15.8	448	7.87	69.8	-	0.127	-	29.2	-	0.004	-
,,	4	,,	17.4	448	7.90	67.0	-	0.111	-	28.3	-	0.005	-
,,	8	,,	19.2	446	8.00	67.7	-	0.106	-	26.5	-	0.004	-
,,	12	,,	18.6	427	7.95	68.1	-	0.108	-	26.7	-	0.004	-
,,	24	03.08.82	15.0	392	7.90	71.8	71.6	0.143	0.102	27.7	27.6	0.022	
,,	36	,,	19.7	445	8.12	71.4	-	0.174	-	26.1	-	0.110	-
,,	48	04.08.82	15.2	446	7.99	74.5	73.2	0.132	0.099	32.5	31.5	0.008	< 0.003
,,	72	05.08.82	16.3	438	8.08	71.3	71.4	0.113	0.098	26.1	26.7	0.004	< 0.003
,,	96	06.08.82	17.0	427	8.04	71.7	72.5	0.124	0.078	24.8	25.4	0.005	< 0.003
,,	120	07.08.82	15.0	445	8.01	73.3	73.3	0.141	0.096	29.4	27.8	0.033	0.017
		$\bar{x}$	-	436	7.98	71.0	72.1	0.129	0.098	27.7	27.6	0.019	-
		S.D.	-	17	0.08	2.7	1.0	0.020	0.012	2.1	2.1	0.032	-

Table 6.6 Aqueous metals (mg l^{-1}), temperature, conductivity and pH in 'Moor Intake Gill' during the five-day transplant period

T = 'total', F = 'filtrable'

6.32 Barium in *G. pulex* during five day transplant from 'Mossy Thorn Gill' (0350) to 'Moor Intake Gill' (0336)

Barium concentrations in *G. pulex* during the five-day transplant are shown in Table 6.7 and Figure 6.3. Table 6.8 compares the barium concentrations in *G. pulex* during the five-day transplant period.

Comments

i) The animals experienced an increase in aqueous barium concentration of about 27.4mg l^{-1}. The concentration of barium in *Gammarus* increased by 166%

ii) The concentration of barium in *Gammarus* appeared to reach an equilibrium after three days

iii) The mean rate of barium uptake over the first three days was 1043µg g^{-1} dry wt day^{-1}

iv) There was no trend of increase in the coefficient of variation over the five days, as was seen in the laboratory uptake study

t(hours)	n	$\bar{x}$	±95%CL	CV(%)
0	5	1920	274	11.5
2	5	2300	305	10.7
4	5	2340	297	10.2
8	5	2470	157	5.1
12	5	2650	441	13.4
24	5	3370	335	8.0
36	5	3640	643	14.3
48	5	4370	319	5.9
72	5	5050	134	2.1
96	4	5070	691	8.6
120	4	5110	613	7.5

Table 6.7 Mean barium concentration (µg g^{-1} dry wt) in *G. pulex* during the five-day transplant period (transplant from 'Mossy Thorn Gill' (0350) to 'Moor Intake Gill' (0336))

Sample size (n), mean ± 95% confidence limits of the mean ($\bar{x}$ ± 95%CL) and coefficient of variation (CV) are given

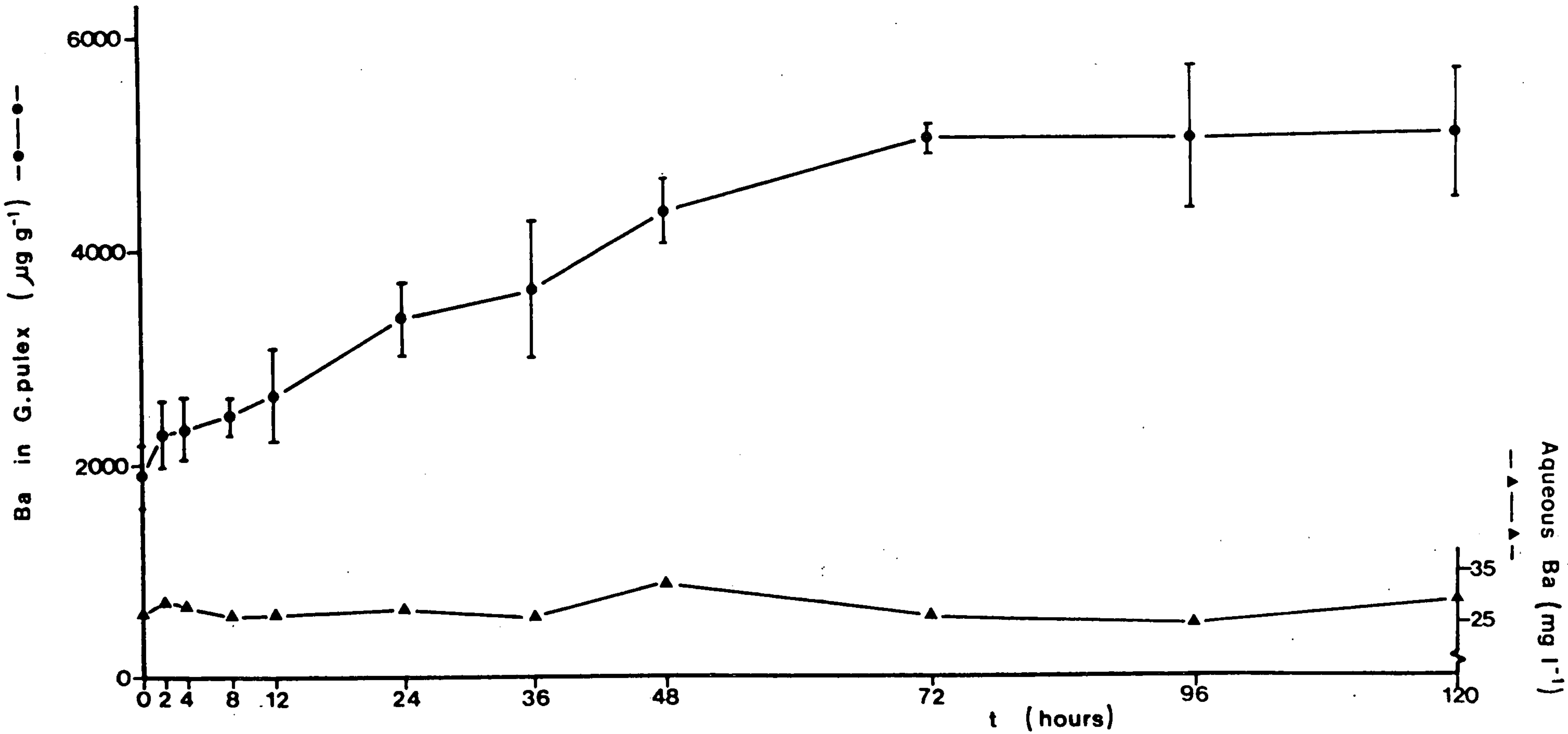


Figure 6.3 Change in barium in *G. pulex* (µg g^{-1} dry wt) and 'total' water (mg l^{-1}) during the five-day transplant period
Vertical bars represent 95% confidence limits of the mean

t(hours)	0	2	4	8	12	24	36	48	72	96	120
0	-	N.S.	*	**	***	***	***	***	***	***	***
2		-	N.S.	N.S.	N.S.	***	***	***	***	***	***
4			-	N.S.	N.S.	***	***	***	***	***	***
8				-	N.S.	***	***	***	***	***	***
12					-	***	***	***	***	***	***
24						-	N.S.	***	***	***	***
36							-	***	***	***	***
48								-	**	**	***
72									-	N.S.	N.S.
96										-	N.S.
120											-

Table 6.8 Comparison between barium concentrations in *G. pulex* over the five-day transplant period (t + 0 - t + 120 hours)

N.S. not significant

* $P < 0.05$ (Analysis of variance and *t*-tests)

** $P < 0.001$

*** $P < 0.001$

6.33 <u>Calcium and zinc in *G. pulex* during the five-day transplant from 'Mossy Thorn Gill' (0350) to 'Moor Intake Gill' (0336)</u>

Calcium and zinc concentrations in *Gammarus* over the five-day transplant are given in Table 6.9 and Figures 6.4, 6.5. Table 6.10 compares zinc concentrations in *G. pulex* during the five-day transplant.

t(hours)	n	Ca			Zn		
		$\bar{x}$	±95%CL	CV(%)	$\bar{x}$	±95%CL	CV(%)
0	5	134000	10200	6.2	121	9	6.3
2	5	139000	23300	13.5	138	35	20.4
4	5	130000	13900	8.6	128	20	12.3
8	5	131000	5790	3.6	109	6	4.6
12	5	127000	14400	9.1	131	25	15.3
24	5	129000	7590	4.8	152	24	12.8
36	5	135000	8820	5.3	145	18	10.0
48	5	127000	11300	7.1	169	12	5.7
72	5	139000	4690	2.7	258	48	15.1
96	4	147000	11600	4.9	157	18	7.3
120	4	138000	9530	4.4	158	25	9.9

Table 6.9 Calcium and zinc (μg g^{-1} dry wt) in *G. pulex* during the five-day transplant period

Sample size (n) mean ± 95% confidence limits of the mean (95%CL) and coefficient of variation (CV) are given

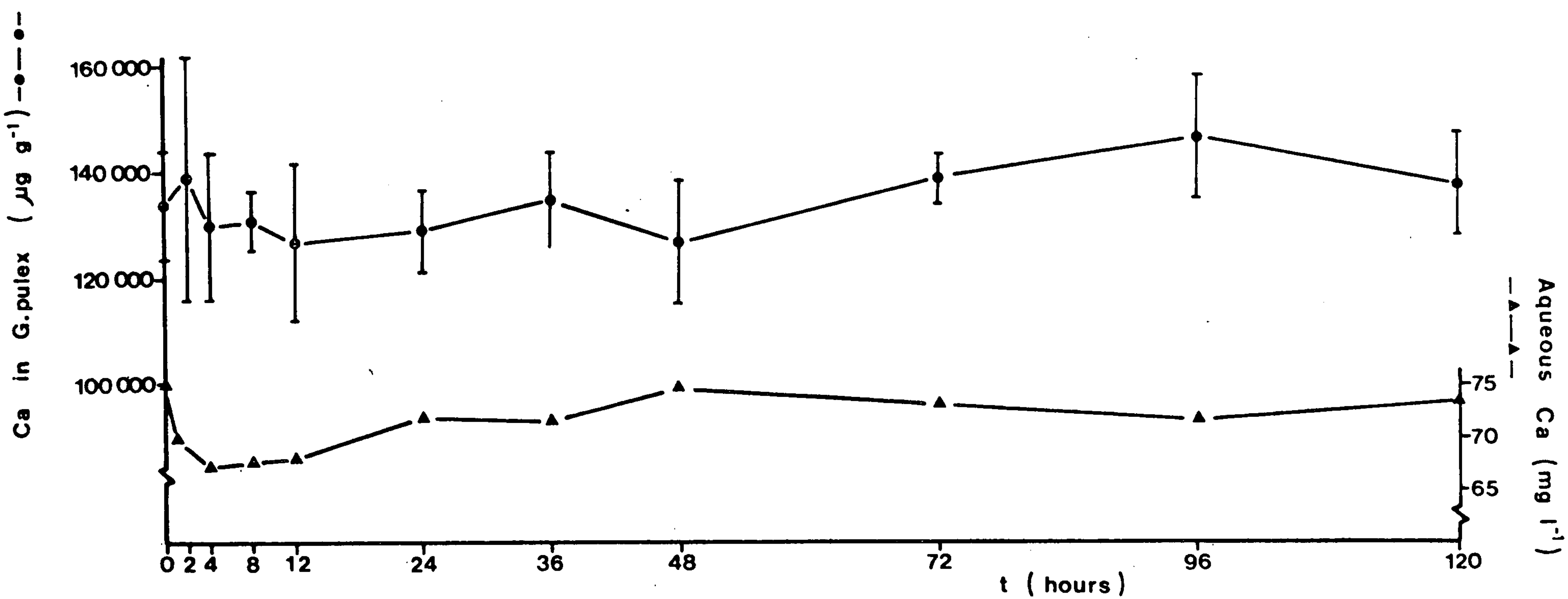


Figure 6.4 Change in calcium in *G. pulex* (μg g^{-1} dry wt) and 'total' water (mg l^{-1}) during the five-day transplant period

Vertical bars represent 95% confidence limits of the mean

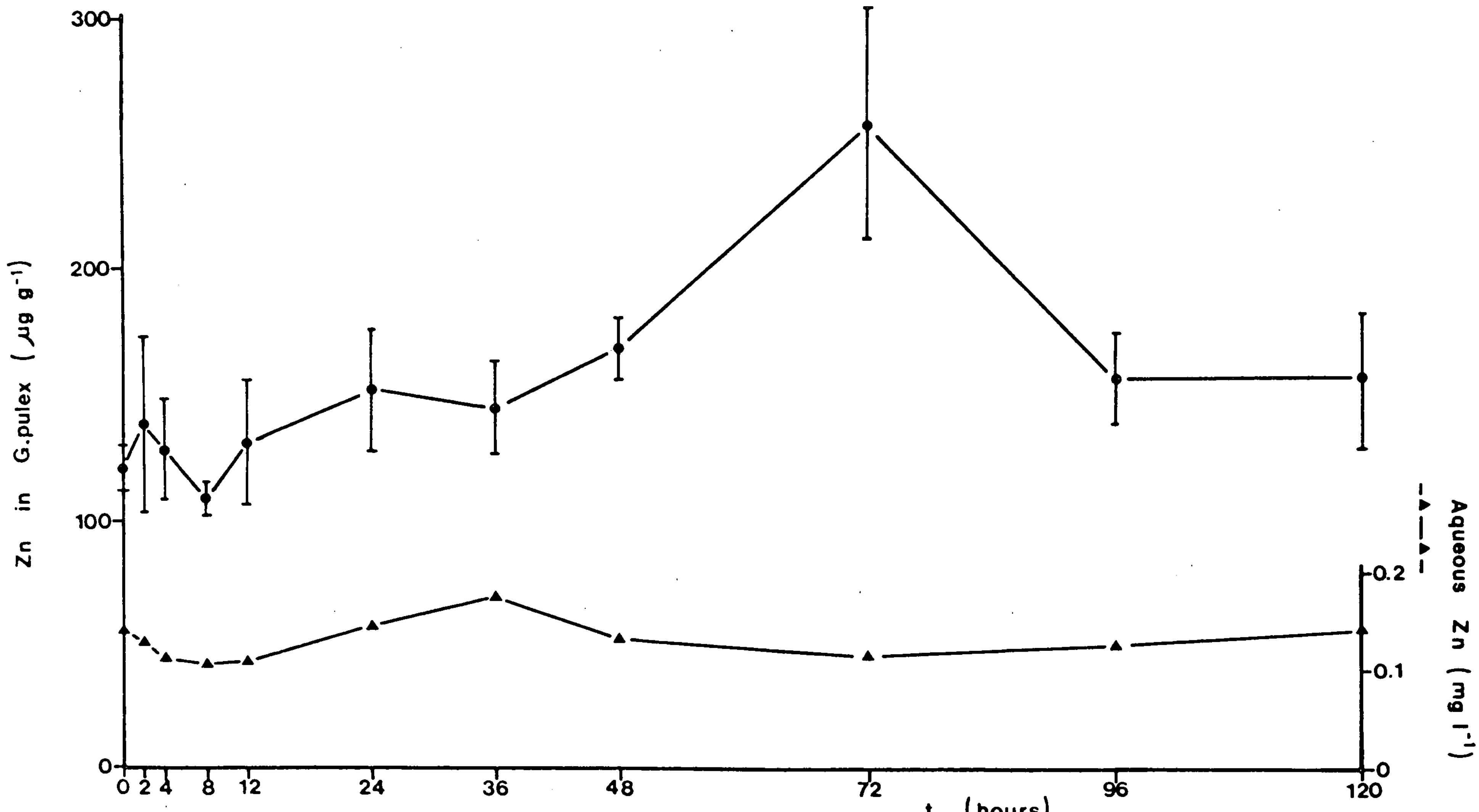


Figure 6.5 Change in zinc in *G. pulex* (µg g^{-1} dry wt) and 'total' water (mg l^{-1}) during the five-day transplant period

Vertical bars represent 95% confidence limits of the mean

t(hours)	0	2	4	8	12	24	36	48	72	96	120
0	-	N.S.	N.S.	N.S.	N.S.	*	N.S.	***	***	**	**
2		-	N.S.	*	N.S.	N.S.	N.S.	*	***	N.S.	N.S.
4			-	N.S.	N.S.	N.S.	N.S.	**	***	*	*
8				-	N.S.	**	**	***	***	***	***
12					-	N.S.	N.S.	**	***	N.S.	*
24						-	N.S.	N.S.	***	N.S.	N.S.
36							-	N.S.	***	N.S.	N.S.
48								-	***	N.S.	N.S.
72									-	***	***
96										-	N.S.
120											-

Table 6.10 Comparison between zinc concentrations in *G. pulex* over the five-day transplant period (t + 0 - t + 120 hours)

N.S. not significant

* $P < 0.05$ (Analysis of variance and *t*-tests)

** $P < 0.01$

*** $P < 0.001$

Comments

i) A doubling of calcium concentration from 'Mossy Thorn Gill' to 'Moor Intake Gill' caused no apparent increase in the calcium concentration of *Gammarus*. *t*-tests after an analysis of variance showed the calcium concentration t + 96 hours to be significantly higher than t + 0, 4, 8, 12, 24, 48 hours, but not significantly higher than t + 2, 36, 72, 120 hours. It is possible that this significant difference may not be biologically meaningful

ii) *Gammarus* experienced an increase in aqueous zinc of an order of magnitude. The concentration of zinc in the animals increased by 113% over the first three days. There was then a sharp drop to a level still significantly above the initial zinc concentration t + 0 (Table 6.10). 48 hours were needed to show a significant increase in zinc concentration from the initial level t + 0 (Table 6.10).

iii) Mortality was observed to be higher between t + 72 and t + 96 hours and t + 96 and t + 120 hours, than during any previous 24-hour period (Section 6.34)

6.34 Mortality

Mortality was not noted during this study. Any dead or dying individuals and cast skins were removed from the cage on each sampling occasion. However, it was noted that in the 24 hours preceding t + 96 and t + 120 hours, mortality was higher than in any previous 24-hour period. About 40 individuals were removed from the cage on each occasion. Since decomposition had already begun, direct comparison could not be made between these individuals and the live samples.

CHAPTER 7

DISCUSSION

7.1 INTRODUCTION

The drainage of the mining area west of Langthwaite, Arkengarthdale, N. Yorks. (Fig. 3.1), particularly Bleaberry Gill, was chosen for this study since it carried elevated levels of heavy metals and barium (Say and Whitton, 1982). A general survey of the aqueous metal concentrations and invertebrate species present revealed suitable sites and species for further work. In the summer, however, the upper, most metal-polluted sites, experienced greatly reduced flow and reduction in the abundance of *Baetis* sp(p)., the group chosen for a comparative study of metal accumulation. Hence these sites were not suitable for use and other streams with elevated metal concentrations were used. 'Mossy Thorn Gill', a tributary to Bleaberry Gill with low aqueous metals, provided a very large number of *G. pulex* for use in transplant studies on barium accumulation. Again sites on Bleaberry Gill, or high barium tributaries in the Fourth Whim area (the area to be reworked) (Fig. 3.1), either had very reduced flow or were dry for much of the summer (e.g. 'Fourth Whim Sike') and so streams draining to the north of the mining area had to be used.

Other studies emerged during the project. Two preliminary studies were needed, one on the concentration of acid required for the digestion of *Baetis* sp(p). and *G. pulex* (Section 2.5) and one on metals in the gut contents of *G. pulex*. It was also interesting to look at calcium and zinc accumulation by *G. pulex* during the field transplant (since 'Moor Intake Gill' had higher concentrations of both these metals).

7.2 BLEABERRY GILL

7.21 Water chemistry

The large variation in aqueous metal concentrations observed along the length of Bleaberry Gill was due mainly to the entry of tributaries of very different water quality.

The stream drains first through Millstone Grit and then over lower Carboniferous rocks (Section 1.32) including limestone. Calcium levels are controlled to some extent by the degree of contact with this limestone bedrock. Concentrations of 20 - 30mg l^{-1} Ca found in the lower reaches are still relatively low, being near the lower limit for hard water (Boycott, 1936). Calcium can be a limiting mutrient for invertebrates, such as molluscs and crustacea (Hynes, 1970), and may therefore affect the invertebrate species composition (Section 7.22).

Bleaberry Gill has a low level of zinc pollution which can be compared with similar studies by Abdullah and Royle (1972), Dowling *et al.* (1981), Abel and Green (1981) and Armitage (1980). The zinc concentration of Bleaberry Gill is still substantially higher than that of streams unaffected by mining activity e.g. Annaside Beck (0371) and Roe Beck (0372) (Table 5.2).

Barium concentrations fluctuated along the whole length of Bleaberry Gill. They appear to depend to some extent on the input from tributaries but, after each decrease in concentration following the entry of a barium poor stream, the level recovered. This would suggest that barium is taken into solution from the substrate or there is ground water seepage into the gill. As there is no literature on levels of aqueous barium in unpolluted streams it was not known to what extent Bleaberry Gill is polluted. The following comparisons were made:

	stream - reach	aqueous Ba (mg l^{-1})
Annaside Beck (Fig. 3.1, Section 3.1)	0371 - 98	0.14
Roe Beck ,, ,,	0372 - 60	0.24
River Wear	0008 - 65	0.32
Bleaberry Gill (Fig. 3.3)	0212 - 20	1.14 - 2.48
Springs and streams draining the Fourth Whim tip complex (Say and Whitton, 1982)		2.86 - 23.5
Moor Intake Gill (Fig. 3.1, Table 4.4)	0336	3.45 - 27.1

It can be seen (Table 4.1) that most of Bleabery Gill is subject to relatively low levels of barium pollution.

Lead concentrations appeared to fluctuate independently of inputs from tributaries, suggesting that the concentration is dependent on other factors such as interactions with the sediments and ground water seepage. Sediments are known to act as a 'sink' for lead (Leland and McNurney, 1974); Pb^{1+} ions precipitate out of solution at pH 6.0 (Dean *et al.*, 1972). Thus changes in the pH of the water may affect uptake from the sediments into solution. The pH of most of Bleaberry Gill was > 6.5 but a lowering of this, by a flush of acidic water, could bring lead into solution. Bleaberry Gill experiences elevated aqueous lead concentrations when compared with similar studies by Abdullah and Royle (1972) and Dowling *et al.* (1981).

The variation observed in pH, conductivity and SO_4^{2-} - S was due partly to the input from tributaries. The observed conductivity values were 2 - 3 times higher than those obtained by Say and Whitton (1982) for similar sites. This is possibly a concentration effect caused by reduced flow during the summer months.

It was possible from these data to choose sites for further study. Sites for the comparative study between metals in water and *Baetis* sp(p). were required to include a range of aqueous metal concentrations. Seven reaches on Bleaberry Gill were used but, since these alone did not give a sufficient range, other streams in the study area had to be included.

should have discussed ecotypes

7.211 <u>Variation in aqueous metal concentrations with time</u>

The observed variation with time in aqueous metal concentrations at selected reaches (Section 4.3, Tables 4.4, 4.5, 4.6) is probably related to changes in water flow. When aqueous metal concentrations were compared with rainfall data (Table 4.5, Fig. 3.5), and hence flow, several trends were noticed. Calcium concentrations were observed to be higher during dry periods and lower after heavy rain, probably due to dilution. Zinc and lead concentrations tended to decrease during dry periods and increase after rain, probably due to increased flushing out of these metals. These data agree with Williams *et al.* (1973) and confirm the observations of the Cornwall River Authority on the River Hayle (quoted by Brown, 1977), but conflict with Burrows (1981), Jones (1940, 1958), Brown (1977), Abel and Green (1981).

The response of these aqueous metal concentrations to increased rainfall and flow must depend to a large extent on the source and availability of the metal. Zinc and lead concentrations were positively correlated at 0212-49 (Section 4.33), while both were negatively correlated with calcium. This is consistent with the observations of the effects of flow on metal concentrations. Zinc and lead may have a common source which is affected similarly by flow. The aqueous barium concentrations were not correlated with calcium, zinc or lead (Fig. 4.2d-f); thus the metal may have a different source or require different conditions before it is brought into solution. Many other variables may be involved.

Plants and animals from Bleaberry Gill and other streams in the area therefore have to experience large variations in aqueous metal concentrations throughout the year (Tables 4.5, 4.6). It is possible that changes in the water flow caused by changes in rainfall may be one of the main factors contributing to this variation.

7.22 Invertebrate species composition

The general composition of the fauna of Bleaberry Gill (0212) was similar to that of other moorland streams e.g. Hynes (1961), Morgan and Egglishaw (1965), Minshall and Kuehne (1969), Arnold and Macan (1969), Armitage (1980), Brooker and Morris (1980a,b), Burrows (1981). Species number and abundance were relatively low; four factors may have contributed to this:

a) rapid variations in discharge

b) water quality

c) low habitat diversity

d) metal pollution

a) Bleaberry Gill experiences fairly rapid increases and decreases in flow, a feature which may reduce both abundance and number of species in the fauna (Minshall and Kuehne, 1969). A decrease in stream flow may increase the number of animals in the drift (Minshall and Winger, 1968) and a sudden increase in flow may then cause the removal of large numbers of these benthic invertebrates. This may also affect the substrate stability. Bleaberry Gill has an unstable substrate with very few permanent areas of deposition. 'Mossy Thorn Gill' (0350) experiences changes in flow that are by no means as large as the main stream, consequently the substrate of 'Mossy Thorn Gill' remains relatively stable. This may be one reason why the stream has a higher species complement.

b) Egglishaw and Morgan (1965) found they could group the streams studied into two groups, differing in species composition and abundance, according to the total cation concentration (> and < 0.4 m-equiv. l^{-1}). Total cations were not measured in the present study but Say and Whitton (1982) found that Bleaberry Gill fell into the chemically poorer category. This may affect animals directly through the specific nutrient requirements of a species, or indirectly through its effect on plant growth or on a prey species. Moulds Old Level (0328) had a much higher level of cations (Say and Whitton, 1982). Chemically it was very similar to 'Mossy Thorn Gill'; it also had a similar fauna, supporting *G. pulex* and flatworms (Say and Whitton).

c) A stable ecosystem with high habitat diversity results in greater species diversity (MacArthur and Wilson, 1967; Patrick, 1975). Bleaberry Gill has a low habitat diversity; the substrate structure is limited with a high proportion of large boulders and stones. It has few areas of slow current and therefore few areas of substrate deposition; thus there are few species from this type of habitat. *Sialis fuliginosa*, an insect characteristic of such depositing substrates, was found at only one site on Bleaberry Gill. Armitage *et al.* (1975) collected a very large number of taxa compared with the present study, Morgan and Egglishaw (1965) and Minshall and Kuehne (1969). Their study area was at high altitude with a rigorous climate but the streams were characterized by a wide range of flow conditions, relatively rich inorganic content, high plant detritus, relatively high pH (6.75 - 8.00) and drained through areas of limestone grassland as well as peat banks. This high habitat diversity resulted in a species rich community.

d) It is possible that the metal concentrations in Bleaberry Gill affect the species composition and/or abundance of the fauna. However there are very few similar studies for comparison.

Some groups of animals were not found, or found in low numbers, in Bleaberry Gill. Two important groups are the molluscs and Malacostraca; other groups are the flatworms, oligochaetes and leeches. Jones (1958) considered molluscs and Malacostraca to be absent because of zinc pollution but suggested that the latter three groups were rare because of the scouring action of mine rubble. Bleabery Gill experiences a low level of zinc pollution and it is unlikely that this limits *G. pulex* distribution, except possibly at the uppermost reaches. Abel and Green (1981) found *G. pulex* and the caddis family Limnephilidae to be absent from high zinc sites (0.45 - 3.68mg l^{-1}, $\bar{x}$ 1.31) and, in laboratory toxicity tests on *G. pulex*, found the 96-hour median lethal zinc concentration to be 2.1mg l^{-1}. Solbé (1977) also found *G. pulex* to be killed within four days exposure to 2mg l^{-1} zinc. It is probable that the zinc concentration has little part in determining the species composition of Bleaberry Gill. Reach 20 had the highest zinc concentration but supported Limnephilidae (c.f. Abel and Green, 1981). It is most probable that *G. pulex* is limited in its distribution by the stability of the substrate. It is known to live in a variety of substrates, from sand to fist-sized stones (Marchant, 1981) but these substrates suggest the presence of more stability than is found in Bleaberry Gill. Also, the majority of the diet of stream dwelling *Gammarus* is known to be allochthonous detritus (Moore, 1975), with small amounts of algae and animal remains (as observed during the present study). Bleaberry Gill has few areas of substrate deposition and little allochthonous detritus.

Molluscs are absent from Bleaberry Gill possibly due to metal pollution (Ortmann, 1909; Carpenter, 1922, 1924; Jones, 1958; Wurtz, 1962). 'Mossy Thorn Gill', the only stream to support molluscs in this study, had low concentrations of heavy metals.

Calcium may be a limiting nutrient for both molluscs and *G. pulex*. Edwards *et al.* (1978) reported that the Malacostraca and molluscs, such as *Ancylus*, occurred only at sites where the mean calcium concentration was > 10mg l^{-1}. Throughout the present study, the calcium concentration was higher than this, on most reaches of Bleaberry Gill, but in winter the concentration may be reduced by dilution (Section 7.211).

There is no literature on the effect of increased barium on species composition and, since so many variables were present, it is difficult to determine any definite effect. Similarly literature is limited on lead and species composition and abundance.

It can be concluded that the invertebrate species composition and abundance are not severely affected by the relatively low level of metal pollution in Bleaberry Gill. Low abundance or absence of species may be a metal pollution effect although other chemical and physical factors may be more important.

7.221 Effect of sampling method on species composition

There are several factors to consider in the interpretation of the species composition of invertebrate samples such as those collected on 10.05.82. The stage of the life cycle varies with the time of the year, thus the species present and their abundance depends on the time of the year. This was seen in the observed 'loss' or reduction of some stonefly groups and 'appearance' of *Ephemerella ignita* in Bleaberry Gill later in the summer. Streams should therefore be sampled several times a year to give a true picture of the species composition. The size of net used for capture is also important. A species may appear to be absent from a stream purely because it is in too small a stage to be caught. The type of substrate sampled also affects the results obtained. A rocky/stony bed is far harder to sample than a gravel/silt bed. The substrate type may prevent capture of certain species. It is possible that this may have been why oligochaetes were not present in this survey. A timed sample may reveal more species and organisms in one type of substrate purely because the animals are easier to find. It is particularly difficult to sample at depth in rocky and stony substrates. A numerical approach should therefore be undertaken with care when the substrates are different.

7.3 METAL CONCENTRATIONS IN *BAETIS* SP(P). IN RELATION TO AQUEOUS METAL CONCENTRATIONS

Freshwater invertebrates may accumulate metals from a large number of sources (Section 1.21). Water is a potential source for all freshwater organisms. This study analyses the relationships between metal concentration in *Baetis* sp(p). and water in an attempt to answer two questions:

a) is water a major source?

b) could this group of animals be used to monitor changes in aqueous metal concentrations?

Baetis sp(p). accumulated substantial concentrations of zinc, barium and lead from Bleaberry Gill (O212) and other metal-polluted streams in the study area. Animals from Annaside Beck (O371) and Roe Beck (O372), streams not affected by mining activity, with low aqueous zinc, barium and lead, had low concentrations of these metals. It can be concluded that elevated levels of metals in the environment give rise to higher concentrations in *Baetis* sp(p)..

A comparison of all the reaches sampled (Section 5.23) gave a significant positive relationship between barium in the water and *Baetis* sp(p).. This may have been weighted by values from 'Moor Intake Gill' (O336) where aqueous barium was very high. However, there was a similar relationship for Bleaberry Gill alone. There was no relationship between the concentration of zinc and lead in water and *Baetis* sp(p).. A relationship such as is shown by barium may indicate water to be an important source of that metal. However, lack of a relationship does not imply that water is not important, since observing a relationship will depend on the rate of response of the animals to fluctuations in aqueous metal concentrations, the quantity of metal in the gut contents (Section 6.1) and the capacity of the animals to eliminate the metal after uptake.

There were notable differences in the zinc, barium and lead concentrations of animals from different streams, suggesting other factors to be operating on metal uptake. Zinc was substantially higher in animals from 'Moor Intake Gill' and Old Gang Beck (O374) than from Bleaberry Gill, Moulds Old Level (O328) and 'Fourth Whim Sike' (O323), even though these latter streams had comparable aqueous zinc concentrations. It is possible that 'Moor Intake Gill' and Old Gang Beck experience flushes of high zinc which result in elevated levels in the animals. Both these streams had higher concentrations of barium and/or calcium; it is possible that these two metals are important in promoting the accumulation of zinc. Similarly lead tissue concentrations tended to be lower at reaches on Bleaberry Gill than in samples taken from streams with comparable aqueous lead. This difference between streams was especially noticeable when barium in *Baetis* sp(p). was compared with aqueous barium (Fig. 5.1c). Animals from 'Mossy Thorn Gill' and Moulds Old Level had unexpectedly high barium concentrations. It is probable that, in this case, water was not a major source of this metal. Section 1.22 discusses possible factors operating to give the observed results.

One factor which may help to explain the elevated levels of barium in animals from 'Mossy Thorn Gill' is the occurrence of

Rhynchostegium riparioides and other mosses. *R. riparioides* was found to contain levels of barium similar to that found in the moss from Bleaberry Gill (pers. comm. C.M. Owen). *Baetis* sp(p). are known to feed on algal growth which covers this type of moss (Hart and Fuller, 1974) and may therefore accumulate the barium from this source. However, this may not completely explain the difference since concentrations of barium in *R. riparioides* from these two streams were about the same, whereas concentrations in *Baetis* sp(p). from 'Mossy Thorn Gill' were 3 - 17 times higher than those from Bleaberry Gill.

Future work would involve the study of more water chemistry variables including the sediments. It is known that the sediments are an important 'sink' for some metals e.g. lead (Leland and McNurney, 1974).

Both the Bleaberry Gill profile study (Section 5.24) and the results from 0212-49 sampled four times (Section 5.25) showed little direct relationship between the concentrations of metals in the water and animals. This suggests that water is not a primary source of these metals or, if it is, that there is some delay in the response of *Baetis* sp(p). to changing metal concentrations or some regulatory mechanism is involved.

7.31 Use of *Baetis* sp(p). as a monitor of aqueous metal concentrations

The present study suggests that *Baetis* sp(p). is not suitable for use as a monitor of zinc and lead but may be of some use in monitoring barium, this depending to a large extent on the stream studied. It is clear that much more work is needed on the biology of this group and its response to known stable elevated concentrations of heavy metals.

A good biological monitor of water quality should be abundant, widespread and robust. It should accumulate metals in proportion to the concentration of the aqueous metal. Water should therefore be the major source of that metal. The animal should also accumulate the metal quickly to reach an equilibrium with the aqueous metal concentration, but then lose it slowly so that elevated metal concentrations will be recorded in the monitor organism some time after the contamination. Such a monitor would therefore show a good relationship with aqueous metal concentrations under conditions of stable water chemistry, but would give results with a large amount of scatter (with tissue concentrations being relatively higher than expected unless the aqueous metal concentration was very high) when collected from water with fluctuating metal concentrations.

7.4 METAL ACCUMULATION BY *GAMMARUS PULEX*

7.41 Effect of gut contents on metal concentration measurements

In the design of the laboratory and field transplant investigations it was necessary to decide whether the animals would be allowed to feed. Since the contribution of the gut contents to the metal concentration measured in the animals was unknown, this preliminary study was carried out (Section 6.1).

The results very clearly showed that the gut contents did contain a very large proportion of the zinc, barium and lead in animals from 'Mossy Thorn Gill' but did not form a significant proportion of the body weight. Therefore a high proportion of the metals in *G. pulex* was provided by a very low dry weight of gut content material. The gut contents also contributed to a large proportion of the variation in the metal concentrations.

Without more preliminary work it would not have been possible to separate the effect of increase due to uptake by the organism and that of metals in the gut contents. It was therefore decided to study uptake of metals from water alone. It is possible that, had the animals been feeding, the metal concentration of *G. pulex* in both laboratory and field transplant studies may have been more than double, and the coefficients of variation would probably have been much higher.

7.42 Barium uptake by *G. pulex* - laboratory study

When exposed to elevated aqueous barium *G. pulex* accumulated the metal to a concentration which was significantly higher than the initial concentration after 36 hours and which stabilized after 2 - 3 days. Uptake from solution may have been by adsorption of the metal on to the cuticle and/or absorption through the cuticular, respiratory and gut surfaces.

The concentration of barium in animals exposed to elevated barium (B), after 24 hours exposure, was significantly higher than that of animals from 'clean' water with no additional barium (A), but, after further statistical analysis (Section 6.211), it was clear that 36 hours were needed to be certain of this difference. Had more samples been taken at each sampling time, a statistically significant increase may have been shown after 24 hours.

The increase in the coefficient of variation over the sampling period, shown by animals exposed to high barium, was possibly due to individual variation in barium uptake. With further exposure to high barium this coefficient of variation may have decreased as a saturation level was reached.

Since the mortality was low throughout the three days and there was no difference between the mortality of animals exposed to high and low barium, it can be concluded that the aqueous barium concentration of 6mg l^{-1} had no toxic effect over this time period.

During this study the dosing solution was not replenished; it was a closed system. The aqueous barium concentration decreased during the three days of study; barium may have been adsorbed onto the surface of the tank and some may have precipitated out through bonding with the 'filtrable' 'Mossy Thorn Gill' water. Uptake by the animals themselves may also have reduced the barium concentration of the system. The results can be compared with those of Kormondy (1965), Kinkade and Erdman (1975), Gillespie *et al.* (1977) and the field transplant study (Section 7.43, 7.44).

The barium concentration in *G. pulex* did not return to its initial level in 11 days after transfer back to 'clean' low barium water (Section 6.22). Some concentrations were very high, possibly due to the presence of individuals with exceptionally high barium; it is possible that the sample included animals which had eaten the carcasses or exuviae of others. Replicate samples should have been taken at each occasion to give a more reliable mean, but this was not possible due to shortage of equipment and time for analysis.

7.43 Barium uptake by *G. pulex* - field transplant study

G. pulex transplanted from 'Mossy Thorn Gill' (0.45mg l^{-1} Ba) to 'Moor Intake Gill' (28mg l^{-1} Ba) accumulated the metal to a concentration significantly higher than the initial concentration after four hours (Table 6.8). This was a much shorter time than that given in the laboratory study, where 24 - 36 hours were needed to give a significant increase.

The mean barium concentration appeared to reach an equilibrium after three days. The laboratory investigation showed a similar result, 2 - 3 days being given as the time needed for an equilibrium to occur. The average uptake rate over the first three days of each investigation was as follows:

laboratory $210 \mu g\ g^{-1}\ day^{-1}$

field $1043 \mu g\ g^{-1}\ day^{-1}$

The mean rate of uptake in the field experiment was therefore about five times greater than in the laboratory. The concentration of barium in 'Moor Intake Gill' was about five times that of the experimental high barium tank.

It can be concluded that, with exposure to a higher concentration of barium, accumulation proceeded at a greater rate to a higher level and that this rate appeared to depend on the aqueous barium concentration. Further work on the response of *G. pulex* to other barium concentrations is needed to confirm this latter statement.

The percentage increase in tissue barium concentrations did not appear to be related directly to the increase in aqueous barium concentration experienced by the animals.

Mortality was high during the 24 hours up to t + 96 and t + 120 hours. Barium may not have been the cause of these deaths, since, if simply accumulating high concentrations of the metal caused death, then it would be expected that the barium concentrations measured at t + 96 and t + 120 hours would have been lower i.e. survivors would be individuals which did not accumulate to a lethal concentration. However, barium may have been taken up equally by all individuals, the more sensitive ones then dying. Another possibility is that the zinc concentration may have contributed to these deaths; this is discussed in Section 7.44. It is also suggested that lead concentrations may also have been important.

More work is obviously needed to isolate the effects of barium, zinc and lead and to study the importance of other variables.

7.44 Further discussion and comparison with the literature

The present study showed uptake of barium to occur when *G. pulex* was transplanted from low to high aqueous barium. The uptake rate appeared to be related to the external barium concentration, as did the final equilibrium concentration which was higher in the higher aqueous barium. The equilibrium concentration attained in 6mg l^{-1} barium was therefore not the limit of accumulation of the animal. Similar equilibria have been observed by Kormondy (1965) who reported an equilibrium of ^{65}Zn uptake after 24 - 48 hours, and Kinkade and Erdman (1975) who showed that concentrations of ^{115}Cd tended towards an equilibrium as the experiment progressed. Both of these studies were carried out in a closed system where the dosing solutions were not replenished. They both noted a rapid initial loss of metal from solution, with the concentration tending to a constant level. The laboratory study (Section 6.2) showed a similar rapid decrease in concentration over the first four hours; after this the concentration appeared to continue to decrease although it fluctuated considerably. The equilibria reported in the above three studies may therefore appear to represent a limit of uptake within the experimental system rather than the limit of metal concentrating capacity of the animals. However, the animals in the field transplant study, which had continuous throughput of high aqueous barium, also appeared to reach an equilibrium, though at a higher level. It can be concluded that there is a limit of uptake of barium by *G. pulex* which may be governed by the aqueous metal concentration.

When the barium uptake rates, for laboratory and field studies, were compared, the rate was greater in the higher aqueous barium.

A similar response (with other metals) was shown by Gillespie *et al.* (1977) with cadmium, and Nehring (1976) with copper, lead and zinc (Section 1.22b).

It is possible, however, that the greater rate of uptake observed in animals transplanted into the higher aqueous barium of 'Moor Intake Gill' (0336) was due partly to temperature, since the temperature of 'Moor Intake Gill' was approximately twice that of 'Mossy Thorn Gill' (0350). As mentioned in Section 1.22, the metabolic rate of invertebrates is positively related to body temperature. An average Q_{10} value of about 2.0 is given for crustacea by Maynard (1960). Schwartzkopff (1955), working with *G. pulex*, found the number of heart beats per minute, for $5^{\circ}C$ acclimated animals, to increase from 100 - 190 over the range 8 - $18^{\circ}C$. This also suggests a Q_{10} of about 2, although it is not certain that heart beat rate and metabolic rate are correlated in such a simple manner (Schwartzkopff, 1955). The following Q_{10}s have been given for *Gammarus* spp.:

species	temperature range	Q_{10}	author
G. limnaeus	0 - $20^{\circ}C$	about 2.0	Krog, 1954
G. oceanicus	5 - $18^{\circ}C$	1.9	Suomalainen, 1958
,,	5 - $15^{\circ}C$	2.4	Halcrow and Boyd, 1967

If metal uptake is an active process one might expect an approximate doubling in the rate of uptake with a $10^{\circ}C$ rise in temperature. In the present study the animals experienced an increase of 6.8 - $11.5^{\circ}C$, from an initial temperature of $8.3^{\circ}C$, but showed a five-fold increase in the rate of uptake. The increase in temperature is therefore unlikely to account fully for the increase in uptake rate.

It has been previously mentioned that the mortality in the 24 hours preceding the t + 96 and t + 120 hour samples in the field study was much higher than in any previous 24-hour period. This could be due to barium toxicity, but two other possible causes are zinc and lead. The increase in mortality coincided with a decrease in the *Gammarus* zinc concentration (Fig. 6.5). This would suggest that death of *Gammarus* with high zinc concentration may have occurred. However, only about 40, or 10% of the animals in the cage died in each 24-hour period; could this 10%, or less than one animal/sample, have had sufficiently high zinc concentration to raise the measured concentration by 100μg g^{-1}, or 63%? Zinc concentrations in 'Moor Intake Gill' were relatively low compared with those found by Abel and Green (1981) and Armitage (1980). Abel and Green (1981) considered *G. pulex* to be sufficiently sensitive to be killed by zinc concentration in the West Allen; sites ranging from 0.04 - 0.56 to 0.65 - 4.15mg l^{-1} ('filtrable' concentrations). On the whole these concentrations were much higher than those of 'Moor Intake Gill'. In laboratory studies they found the 96-hour median lethal concentrations to be 2.1mg l^{-1}, and Solbé (1977) showed that *Gammarus* was killed within four days by exposure to 2mg l^{-1} zinc. These concentrations are an order of magnitude higher than those of 'Moor Intake Gill'.

Spehar *et al.* (1978) found lead to be acutely toxic to amphipods, showing greater than 50% mortality at 0.136mg l^{-1} after four days exposure and 60% mortality after a 28 day test in 0.032mg l^{-1} (nearly all the animals died at higher concentrations). Lead concentrations varied in 'Moor Intake Gill' throughout the study period (Table 6.6). It can be seen that the lead concentration was mostly in the region of 0.004 - 0.008mg l^{-1} but on three occasions the 'total' lead concentration was higher, at a level where it could be toxic to *Gammarus*.

The high mortality in the fourth and fifth day of study may be due, at least in part, to the aqueous zinc and/or lead concentration. Some such toxicity effect may also have prevented *Gammarus* from colonizing 'Moor Intake Gill', since other stream characteristics e.g. substrate and flow were found to be suitable.

7.45 <u>Use of *G. pulex* as a monitor of barium pollution</u>

Section 7.31 reviews some of the requirements of a good monitor. *G. pulex* fulfills the first requirements in that it is a widespread, abundant and robust animal. The present study suggests it may be suitable for detection of high aqueous barium pollution or lower level contamination of one day duration or more, but would be unsuitable for detection of short term, low barium pollution. A change in the sampling method used, by increasing the sample number and sample size, would increase the accuracy of the mean values obtained and so make it possible to show a significant increase in barium concentration of *G. pulex* after a shorter exposure time.

The laboratory study (Section 6.22) indicates that *G. pulex* does release barium slowly after uptake, therefore allowing detection some time after the high barium contamination.

It is clear that more information is needed on the biology of *G. pulex* and its response, under controlled conditions, to elevated aqueous barium concentrations, before it could be used in monitoring barium pollution. For example, it is not known in what tissues the barium accumulates. If it is incorporated in the exoskeleton (perhaps by competition with calcium), as was suggested by the study on *Baetis* sp(p)., Section 5.25, it may be lost at moult. This would limit the length of time high barium concentrations would be measurable in the animals.

Further work is needed on the barium concentration in *G. pulex* before and after moult; it may also be possible to stain sections of *G. pulex* specifically for barium (Waterhouse, 1951; Windholtz, 1976).

SUMMARY

1. Bleaberry Gill experienced elevated levels of barium and lead, and, at most reaches, a low level of zinc pollution. The concentrations of these metals in 'total' water varied substantially down the stream, due mainly to the entry of tributaries of very different water quality. Ranges of 'total' aqueous metals (mg l^{-1}) experienced in Bleaberry Gill over the study period were:

zinc	0.030 - 0.26
barium	0.44 - 2.30
lead	0.016 - 0.090

2. Aqueous metal concentrations also varied with time. The variation in calcium, zinc and lead observed at one reach (0212-49) was related to the flow rate; a decrease in the flow tending to cause a decrease in aqueous zinc and lead and an increase in aqueous calcium.

3. The invertebrate fauna of Bleaberry Gill was characteristic of such 'unstable' upland streams. Absence of species or reduction in abundance could be due to metal pollution but many other physical and chemical factors may also be involved.

4. Elevated levels of zinc, barium and lead in the environment gave rise to elevated concentrations in *Baetis* sp(p).. Metal concentrations in animals from Bleaberry Gill followed the pattern Zn > Ba > Pb.

5. There was a positive linear relationship between barium in *Baetis* sp(p). and water, both when all sites were compared ($P < 0.01$) and for Bleaberry Gill alone ($P < 0.001$). No such relationship was found for the other metals studied.

6. There were notable differences in the zinc, barium and lead concentrations of animals from different streams, suggesting other factors to be involved in metal uptake. This difference was particularly noticeable with barium.

7. It is suggested that *Baetis* sp(p). is not suitable for use in monitoring zinc and lead pollution, but may be of some use in monitoring barium.

8. Gut contents in *G. pulex* from 'Mossy Thorn Gill' provided a substantial proportion of the zinc (33%), barium (53%) and lead (60%) measured and also accounted for a large proportion of the variation in metal concentration between samples (zinc 50%, barium 60%, lead 76%).

9. When exposed to an elevated barium concentration (6mg l^{-1}), under laboratory conditions, *G. pulex* accumulated the metal to a concentration significantly higher than the initial concentration after 36 hours and reached an equilibrium concentration after two to three days.

10. When exposed to 28mg l^{-1} aqueous barium in 'Moor Intake Gill', *G. pulex* accumulated the metal to a concentration significantly higher than the initial concentration after four hours and reached an equilibrium concentration after three days.

11. Exposure of *G. pulex* to a higher concentration of barium caused accumulation to proceed at a greater rate to a higher equilibrium concentration. This rate appeared to depend on the external aqueous barium concentration.

aqueous barium (mg l^{-1})	rate of uptake (µg g^{-1} day^{-1})
28	1043
6	210

Since *G. pulex* in 28mg l^{-1} barium accumulated to a higher equilibrium level it is clear that that attained in 6mg l^{-1} barium was not the limit of the metal concentrating capacity of the animals.

12. *G. pulex* may be useful as a monitor of high aqueous barium pollution or lower level contamination of one day duration or more. It may not be possible to use this species for the detection of short term, low barium pollution.

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