Effects on Gammarus of Sediments Impacted by Watercress Farms

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EFFECTS ON *GAMMARUS* OF SEDIMENTS IMPACTED BY WATERCRESS FARM EFFLUENT

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SUMMARY

Concern has been expressed over the absence of Gammarus, a characteristic chalk stream invertebrate, from streams receiving discharge from watercress farms. It has been suggested that this may be in part, or in whole, due to the release of zinc, used to control root infections in the watercress, in particulate and dissolved form, from watercress beds.

Two linked studies were conducted to investigate the effects on Gammarus of sediments derived from a watercress farm discharge, and the possible role of zinc contamination in any observed effects.

Gammarus were either exposed for four and eight weeks to sediment cores from five sites covering a 3.5 km stream segment downstream of the farm, or for 10 days to leaf material experimentally contaminated with zinc. Response was measured in terms of behaviour, feeding rate and change in body weight.

Sediments were found to be only marginally capable of supporting Gammarus. Zinc-contaminated food material was found to significantly reduce feeding rate (by 25-50%) at concentrations (334-4160 mg/kg Zn) comparable to those measured in leaf litter and sediment samples from the five study sites (121-559 mg/kg Zn). The level of leaf litter contamination at 3 of the 5 sites would be sufficient, on the basis of those results, to exert an adverse effect on Gammarus. Concentrations were experimentally achieved in leaf tissue by exposure to dissolved zinc concentrations of the order measured in a single autumn water sample and reported in previous studies for the October-March period in receiving streams. EQS values for dissolved zinc in waters of similar hardness to the stream studied are approximately three to ten times higher than that required to contaminate leaf tissue to 'effect' levels.

Responses were inferred to be behavioural rather than due to direct toxicity, but were nonetheless of a magnitude sufficient to suggest that zinc contamination of sediments makes a substantial contribution to the absence of Gammarus from streams receiving watercress farm discharges.

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SECTION 1 - INTRODUCTION

The watercress industry in the UK discharges dissolved and particulate zinc to receiving waterways as a consequence of the use of the dissolved metal to control root infections. The administration of dissolved zinc takes place between October and March, but zinc accumulated in the growing beds may be released in particulate form during bed-cleaning operations between April and September.

Studies carried out by the FBA (Casey *et al* 1988) have demonstrated that bed-cleaning operations may lead to the release of particulate zinc at transient concentrations of up to 30 mg/l. Particulate vegetable matter and finer bed sediments may also have a marked physical impact, burying natural stream sediments and leading to the colonisation of the receiving habitat by opportunistic and silt-tolerant species. Physical occlusion of natural sediments may be particularly important in smaller receiving streams, where watercress farms make a major contribution to both organic matter input and to freshwater flow.

Concern has been expressed over the absence of the amphipod *Gammarus pulex* from many receiving streams for distances of several kilometres from the point of farm water discharge. *Gammarus* is regarded as a species sensitive to environmental contamination, and as a typical member of chalk stream invertebrate communities. It is also a major food item for fish.

It has been suggested that zinc, as the major known contaminant discharged to affected streams, may be responsible for the absence of *Gammarus*. A variety of possible explanations may be invoked, but such explanations need to take account of the annual variations in the quantity and form of zinc discharged. In outline, the following possibilities have been identified:

a) acute or chronic toxicity of dissolved zinc (winter);
b) acute or chronic toxicity of particulate zinc (summer);
c) acute or chronic toxicity of sediments (winter and summer);

d) substrate unsuitability (sedimentation, occlusion);
e) food unpalatability or unavailability;
f) avoidance of contaminated water or sediment (drift);
g) competition with species colonising areas of high sedimentation.

Consultation with NRA Southern Region identified a need to assess the contribution that sediment contamination might make to the absence of *Gammarus*. Sediments are present throughout the year, whereas releases of dissolved and particulate zinc are confined to alternate halves of the year; the accumulation and persistence of zinc in sediments, together with a physical alteration of the habitat, might therefore provide an explanation for the observed elimination of *Gammarus* populations.

The aim of the present study was to investigate the effects of zinc-contaminated stream sediments and food material on the rate of feeding in experimental populations of *Gammarus*. Feeding rate measurements have been demonstrated to be a sensitive index of sublethal toxic and behavioural response in a number of species (Bayne *et al* 1985, Calow; pers comm), and were appropriate in the present instance since published studies (WRc 1984) indicated that acute lethal effects would not be anticipated at the dissolved and particulate zinc concentrations reported for streams receiving watercress discharges.

SECTION 2 - MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

In consultation with NRA Southern Region, a watercress farm at Abbots Ann was chosen for study (Figure 1). Effluent from this farm is discharged into an artificial channel which joins Pilhill Brook approximately 1 km downstream of the farm. Five sites, between the farm and the confluence of Pillhill Brook with the R Anton, were selected for investigation (Figure 1, Table 1). Site 1, on the Pillhill Brook adjacent to the farm, was free of influence by the farm discharge;

site 2, immediately outside the farm boundary in the artificial channel, was expected to reflect the maximum impact of the farm discharge. The total length of stream within the study area was approximately 3.5 km, and previous visits by NRA biologists had established the absence of Gammarus at all sites including that immediately upstream of the confluence with the R Anton.

The study comprised two separate but co-ordinated investigations:

a) the investigation of possible toxic and behavioural effects on Gammarus of exposure to sediment cores from each site; and

b) the investigation of possible toxic and behavioural effects on Gammarus of exposure to leaf material contaminated with zinc at a range of concentrations.

These experiments were intended to determine whether Gammarus could survive in contact with sediments largely derived from watercress farm discharges, whether such exposure led to persistent sublethal effects, and whether exposure to zinc-contaminated food per se might account for any effects observed as a consequence of sediment exposure.

2.2 EXPERIMENTAL ANIMALS

Adult Gammarus of between approximately 5 and 10 mg dry weight were obtained from an uncontaminated stream in S Wales, and transported with minimum delay to the laboratory. Healthy specimens were transferred to a 20 l glass tank of flowing groundwater (500 ml/min, pH 7.9-8.1, hardness c 250 mg/l, temp 12-13 °C). These animals were allowed to acclimate to laboratory conditions for two weeks, during which period they were observed daily for mortality and fed ad lib on rehydrated alder leaves.

2.3 SEDIMENT EXPOSURE STUDY

2.3.1 Collection of sediment cores and water samples

Sediment cores were collected from all sites on 27 September 1989. Forty-four cores were obtained at each location (Figure 1); forty of these were used in subsequent experiments, and four were frozen immediately upon return to the laboratory for subsequent fractionation and chemical analysis.

Cores were taken from the accumulated fine sediment which predominated at all sites. Eight-centimetre lengths of food-grade ABS pipe of 6.5 cm internal diameter were inserted to a depth of five centimetres in the sediment, and a tightly-fitting polythene lid placed on top, trapping overlying water and ensuring a good internal 'vacuum'. A flat-based polythene scoop was then carefully inserted under the core, and the undisturbed section gently lifted from the water. The core was then transferred to a sheet of thin polythene placed on a flat board by sliding the pipe section smoothly off the scoop onto the sheet. The polythene sheet was securely attached to the pipe section using a nylon cable tie, and the whole core sample transferred to a food-grade polythene tray. These trays were used for transportation and for later experimentation, thus avoiding excessive disturbance of the core structure. Examination of cores following return to the laboratory showed that no disturbance had occurred and that the core structures were intact.

At each site, a single 100 ml water sample was collected. A clean polythene collection bottle (500 ml) was rinsed three times in ambient water before a final sample was taken and decanted carefully into a clean 100 ml polythene bottle containing 0.5 ml nitric acid as preservative.

2.3.2 Physical and chemical analysis of sediment and water samples

(a) Water samples

Water samples were analysed for total zinc by Inductively Coupled Plasma Atomic Emission Spectrometry. The limit of detection was 0.003 mg/l Zn.

(b) Sediment samples

The four replicate cores from each site were defrosted and pooled before physical fractionation. Prior to sieving, all identifiable leaf litter was removed and dried at 80 °C for 48 h. Dried leaf material was transferred to a desiccator, cooled, and weighed. The remaining material was wet-sieved through nylon mesh of 250 and 125 µm pore size into three fractions. Each fraction was dried and weighed in the same manner as the leaf material.

All fractions, including leaf material, were digested in aqua regia, following the SCA method (1986), and analysed for zinc by Flame Atomic Absorption Spectroscopy.

2.3.3 Exposure of *Gammarus* to sediments

Upon return to the laboratory, sediment cores were randomised within the polythene trays. Each tray contained 20 cores, and two trays were used per site. Sites were not randomised amongst trays, to avoid cross-contamination. Nylon collars of 2 mm mesh were secured to each core with nylon cable ties; these collars protruded above the rim of each core by approximately 8 cm. Groundwater was added to each tray to a depth of 3 cm above the rim of the cores, or 5 cm above the sediment surface. The nylon mesh collars thus projected 5 cm above the water surface to prevent the escape of animals subsequently added to each core. The mesh size was large enough to allow the free circulation of water, which was promoted by the continuous aeration of the water in each tray by means of lengths of perforated polythene tubing. Air was supplied at constant temperature from an oil-free pressure reservoir.

Since the toxicity of the sediment samples and the suitability of sediment particles as food were not known, each site was divided into four treatments. Half of the cores were allocated to animals which were to be exposed for eight weeks, and half to animals exposed for a shorter period of four weeks. This allowed for the possibility that acute toxicity might become apparent over the longer period. For each exposure period, half of the cores were allocated to animals receiving a supplementary addition of alder leaf discs which had been acclimated in the receiving stream in proximity to the sites of sediment collection for 10 days. This leaf material was recovered at the time of sediment collection and was presumed to be in equilibrium with ambient dissolved zinc concentrations.

Cores were assigned to treatments using random number tables.

The test was initiated by withdrawing Gammarus in groups of 20 from the stock tank, and assigning them, using random numbers, to cores. In this way, any bias introduced by the effects of individual vigour on probability of capture from the stock tank would not be reflected in the distribution of animals between treatments.

The trays were maintained in a constant temperature room (12-13 °C) with natural photoperiod and simulated dawn and dusk. The water was changed twice a week; a residual depth of 2 cm was maintained above the surface of each sediment core when the trays were drained.

Water quality was monitored daily, and temperature, pH and dissolved oxygen (DO) recorded.

Each individual core was examined daily, and the presence, absence, moulting, death and behaviour of each experimental animal recorded. Behaviour was classified as either in contact with, or not in contact with, the sediment surface. The top few millimetres of each core were gently raked with the tip of a cable tie to determine whether non-visible specimens had burrowed.

For animals allocated to the supplementary feeding treatments, four leaf discs were added initially to each core, and observations made twice-weekly to assess whether the previous ration had been consumed. When consumption was apparent, the amount perceived to have been consumed since the last addition was replaced. The leaf discs acclimated in the field were held dry, and re-hydrated approximately four days in advance of use.

2.3.4 Feeding rate measurements

After 4 and 8 weeks exposure to sediments, the feeding rates of 'fed' and 'unfed' animals were measured. In each case, 20 Gammarus (10 fed, 10 unfed; originally assigned at random to cores and treatment) per site treatment were transferred to 250 ml polythene feeding vessels. Random number tables were used to assign animals to vessel positions on a flat, evenly-lit surface adjacent to that on which the sediment-exposure trays were positioned. The vessels were filled with groundwater from the same source as supplied to the stock tank and the sediment exposure trays. Nylon mesh of 1 mm aperture divided each vessel horizontally 3 cm from the bottom. This mesh allowed faeces produced during feeding to drop through, thus preventing re-ingestion.

Each Gammarus was supplied with four 1 cm-diameter alder leaf discs. These discs were prepared from re-hydrated leaves stored from the previous year's abscission. Leaf discs were dried at 60 °C, cooled, and weighed to ±0.01 mg before storage in a dessicator. Discs were rehydrated 4 days before use. Care was taken during handling leaf material to avoid damage which might lead to loss of weight.

For each set of measurements (4 and 8 weeks), 5 additional vessels without animals also received 4 leaf discs each. These served as controls for changes in leaf mass not attributable to Gammarus activity.

The animals were held in the feeding chambers for 6 days, with daily observations for mortality and moulting; the number of remaining leaf discs in each vessel was counted during observation, and more leaf discs added if necessary.

After 6 days, the animals were removed from the feeding vessels and transferred to pre-weighed foil boats. They were crushed lightly within these boats, and then dried at 60 °C for 24 h. Dried animals were cooled in a dessicator and subsequently weighed to ±0.01 mg.

Leaf material remaining in the feeding vessels was carefully removed to pre-weighed foil boats. Material deposited on the vessel bottoms was examined, and any small leaf fragments which had passed through the mesh were separated from the faeces and added to the material already removed. The leaf material was dried at 60 °C for 24 h, cooled and weighed to ±0.01 mg.

The energy consumption of each animal in J/mg/d was calculated as follows:

$$E = \frac{21.552((I*C)-F)}{D*W}$$

where E = energy consumed (J/mg/d)
I = initial dry weight of leaf discs added (mg)
F = final dry weight of leaf discs added (mg)
C = control correction factor (final/original leaf weight)
W = dry weight of animal (mg)
D = number of days of test
21.522 = energy content of inoculated alder leaf (J/mg)

2.4 DIETARY ZINC STUDY

The effect of dietary zinc on feeding rate was assessed by incubating experimental animals with zinc-contaminated leaf material. One-cm diameter leaf discs were immersed in ZnCl solutions of 0, 21, 135, 900 and 6000 µg/l. The zinc solutions were prepared from a 210 mg/l stock in acid-cleaned 5 litre glass tanks. During immersion of the leaf discs, the solutions were gently stirred twice per day. After 48 h, leaf discs were removed, dried and re-weighed before storage in a dessicator. Samples of approximately 20 mg per treatment were dried at 80 °C and analysed as described above for sediment fractions.

The zinc solution concentrations were selected to provide a semi-logarithmic series, and it was assumed that the leaf material would take up approximately 8% of the available metal. This assumption was based on observed zinc losses from solution to leaf material in a previous WRc study (unpublished).

The measurement of feeding rates followed the methods described above, with the following differences:

a) duration was increased from 6 to 10 days
b) 20 animals were used per treatment
c) each animal was provided with 6 leaf discs

After termination of the experiment, the remaining leaf material was pooled by treatment, dried at 80 °C, and analysed for zinc.

SECTION 3 - RESULTS

3.1 SEDIMENT AND WATER ANALYSES

3.1.1 Water

Total zinc concentrations in stream water samples ranged from below the detection limit (<0.003 mg/l) at sites 1 and 4 to 0.027 mg/l at site 2 (Table 2). Concentrations were significantly above the detection limit at both sites 3 (0.008 mg/l) and 5 (0.01 mg/l). Although concentrations were highest immediately below the watercress farm, there was not a monotonic decline with distance.

3.1.2 Sediments

Zinc concentrations were generally higher in sediment fractions of samples from sites 2-5 than from site 1 (Table 3, Figure 2). Site 1 fraction concentrations were in the range 121-162 mg/kg, while those from the remaining four sites were mostly in the 300-400 mg/kg range, with values of up to 853 mg/kg and 559 mg/kg in leaf litter from sites 4 and 5 respectively. At all sites except site 5, zinc concentration increased with increasing fraction particle size.

Since the weight of each fraction was recorded, it was possible to calculate the zinc concentration for the whole sediments (Figure 2) and to estimate the distribution of total zinc between fractions (Table 4, Figure 3). Total zinc was lowest at site 1, highest at site 4, but in fact varied relatively little between sites 2, 3, 4 and 5. The distribution of zinc was biased heavily towards the larger size fractions, with from 44-67% present in the >250 μm fraction in sediment from sites 1, 2, 3 and 4. This pattern was not repeated at site 5, where the highest percentage of zinc was found in the 125-250 μm fraction. Leaf litter did not account for more than 10% of the zinc present at any site, and at sites 3 and 5 represented 1% or less of the total zinc. The bias of zinc distribution was largely accounted for by the physical predominance of the larger size fractions (Table 5, Figure 4).

The percent volatile solids ('organic') content of sediment fractions increased with increasing fraction size at all sites (Table 6, Figure 5); values ranged from 0.9% in the <125 μm fraction in sediment from site 4 to 31.5% in the >250 μm fraction in sediment from site 2. Percent volatile solids appeared to peak at site 2, immediately downstream from the watercress farm, and to decline at sites further downstream.

3.2 SEDIMENT EXPOSURE STUDY

3.2.1 Water quality

Maximum and minimum values of DO, temperature and pH for all treatments are summarised in Table 7. Temperature ranged from a minimum of 9.9 to a maximum of 12.9 °C during the course of the experiment. DO varied between 81% saturation and 100% saturation, while pH varied from 8.11 to 8.57. The ranges observed on any one day were considerably less than the overall ranges indicated above.

3.2.2 Mortality, moulting and loss

The experimental system successfully maintained all but a few animals in proximity to the sediments throughout the study period. Of the 40 amphipods in each treatment, no more than 3 died (Table 8) or 4 escaped. A total of 11 animals died and 10 escaped during the 8 weeks of exposure, and these losses were distributed without bias amongst the treatments.

One or two animals in each treatment moulted during the 8 week period (a total of 7 individuals), suggesting a very low rate of growth and production.

3.2.3 Behaviour

Behaviour was assessed as the percentage of animals in contact with the sediment (either on the surface or buried) during each observation period. During 8 weeks' exposure, 19 observations of 'fed' animals were made (Table 9, Figure 6). Overall, a minimum of 45% and a maximum of 100% were observed in contact with sediment in cores from any one site. Averaged over time, 84% were in contact with sediments from site 1 and 71% with sediments from site 3. There was a wider range of variation between times (averaged over sites) than between sites (averaged over time); a maximum of 96% during observation 5 versus a minimum of 65% during observation 13 (Table 9).

At most times for all sites, a high proportion of animals were in contact with sediment. Since added leaf discs were rapidly incorporated into the surface sediments, this material did not provide a refuge from contact.

For animals recorded as not visible, examination of the observation records showed that the same individuals were repeatedly involved. In all, only 26 animals burrowed consistently into the sediments, and these accounted for the overwhelming majority of the category 'not visible but present'. These individuals were not distributed between treatments with any detectable bias.

3.2.4 Feeding rate measurements

Mean feeding rate, expressed in energy terms and corrected for individual Gammarus dry weight, was calculated for each treatment group (four and eight weeks exposure, with and without supplementary feeding). The effects of site, food and time treatment were examined by factorial Analysis of Variance (ANOVA), followed by significance tests using either Dunnett's test (for comparison of treatment means with 'reference' mean) or Tukey's test (for multiple comparison of means) as appropriate. The factorial design permitted the evaluation of possible interactions between site, food and time effects. Effects were considered significant at a probability of 0.05 or less. Mean dry tissue weight was calculated for each treatment group and analysed similarly.

After four weeks' exposure, feeding rates ranged from 5.28 to 10.22 J/mg/d in fed animals exposed to site 4 and site 3 sediments respectively, and from 7.78 to 11.43 J/mg/h in unfed animals exposed to sediments from sites 5 and 2 respectively (Table 10, Figure 7). The only significant difference (Tukey's test, $p<0.05$) within 'feeding' categories was between fed animals associated with sediments from sites 3 and 4. In a restricted comparison between the reference site and other site means (Dunnett's test), only sites 3 and 4 (fed) differed significantly ($p<0.05$). There was no significant interaction between site and food. Feeding rates did not differ significantly between 'unfed' treatments ($p>0.05$), nor between any site pair or between 'fed' and 'unfed' treatments overall.

After eight weeks' exposure, feeding rates were 5.39 to 7.27 J/mg/d in fed animals, and 6.20 to 8.30 J/mg/d in unfed animals (Table 10, Figure 8). There were no significant differences ($p>0.05$) between any treatment groups, and no significant interaction between the main effects.

ANOVA was also carried out on all data from both periods combined. This revealed that there was a significant effect of time for unfed animals ($p<0.05$, Tukey's test), but not for fed animals. The former showed a decline in feeding rate between four and eight weeks, largely as a consequence of the somewhat higher feeding rates observed in unfed animals at four weeks. When the combined data were analysed for site effects, the only significant ($p<0.01$) difference observed was between fed animals associated with sediment from sites 3 and 4.

3.2.5 Changes in body mass

Mean dry weights were determined for all treatment groups (Table 11). A significant effect of food was apparent after four weeks ($p<0.02$) and eight weeks ($p<0.001$). In both cases, fed animals were heavier than unfed animals. Whilst fed animals generally increased in weight between four and eight weeks, over the same period unfed animals lost weight (Table 11, Figure 9). There was no significant interaction between the main effect (site, food, time) terms.

3.3 DIETARY ZINC STUDY

3.3.1 Leaf zinc concentrations

Experimental leaf material was pooled by treatment, dried and analysed as described above for field samples. The measured concentrations were 230, 334, 391, 1050 and 4160 mg/kg (Table 12); this compared with a range of between 121 mg/kg (site 1, reference) and 559 mg/kg (site 5) measured in field samples of leaf litter.

3.3.2 Feeding rate measurements

Feeding rates were between 1.41 and 2.74 J/mg/d (Table 12), and were thus considerably lower than those observed in sediment-exposed animals. There was an approximately logarithmic decline in feeding rate with increasing concentration (Figures 10 and 11). One-way ANOVA followed by Dunnett's test (restricted comparison of control with treatment means) indicated feeding rates at food Zn concentrations of 334-4160 mg/kg were significantly lower than those at 230 mg/kg. Response was most marked at lower concentrations, where a relatively small increment in leaf Zn (230 to 391 mg/kg) effected a similar reduction in feeding rate as did an 11-fold larger increase at higher concentrations.

3.3.3 Changes in body mass

Mean dry weights were between 6.33 mg (230 mg Zn/kg treatment) and 7.43 mg (391 mg Zn/kg treatment). This difference was not statistically significant ($p>0.05$, Tukey's test).

SECTION 4 - DISCUSSION

4.1 WATER AND SEDIMENT CHARACTERISTICS

The highest water zinc concentration measured in this study was at the lower end of the range reported by Casey et al (1988) for watercress farm outflows during winter dosing of zinc. The present value was, however, based on a single 'spot' sample, and was collected at a time when active bed-cleaning was in progress. Nevertheless, no watercress debris was included in the sample, and the result points to the possibility that zinc in water may be a problem which persists throughout much of the year.

The physical and chemical characteristics of the sediment samples were similar to some, but not all, of the observations reported by Casey et al (1988), and indicate that the sediments were not of 'natural' origin. Larger size fractions predominated, and the tendency for these fractions to be associated with higher 'organic' and zinc content runs counter to the general tendency, in silty sediments, towards higher 'organic' and contaminant levels in the finest fractions. Elevated zinc concentrations in sediments, and especially in leaf litter, from sites 4 and 5 point to possible additional inputs of zinc downstream of the Abbots Ann watercress farm, and indicate a strong spatial persistence of contamination.

4.2 RELATIONSHIP BETWEEN EFFECTS OF SEDIMENT EXPOSURE AND SEDIMENT CHARACTERISTICS

There was no apparent systematic relationship between feeding rate or dry weight and the concentrations of zinc measured in whole sediments or fractions of sediments. Feeding rates were, in general, similar to those estimated for unstressed Gammarus of similar size by Nilsson (1974) and Sutcliffe et al (1981). There was thus no evidence of a persistent sublethal toxic effect following removal from sediment exposure, and it is inferred that contaminant availability from the sediments was insufficient for accumulation to exceed toxic thresholds.

Whilst post-exposure feeding rates did not differ systematically between sites, it is clear, from the changes in dry weights observed, that actual ingestion rates during sediment exposure were markedly reduced, especially in 'unfed' treatments where the sediment cores were the only source of food. Overall, 'fed' animals gained an average of 0.8 mg during the second four weeks, and 'unfed' animals lost 0.6 mg during the same period. These values are equivalent to energy gains or losses of about 20 and 15 J respectively. Either metabolic costs for experimental animals were high, or sediment characteristics at all sites were not well-suited to the requirements of Gammarus. The latter seems more probable, since the post-exposure feeding rates reported do not suggest that the animals were under toxicological stress.

The behaviour of experimental animals, as reflected in the number observed in contact with sediment, indicated that the majority of individuals preferred contact with sediment to being exposed to the level of direct illumination required for observation. Variation in percentage in contact was more apparent between times than between sites. This supports the inference above, that sediments were not directly toxic.

4.3 EFFECTS OF FOOD CONTAMINATION

The effects of dietary zinc on feeding rate were statistically significant even between the two lowest concentrations presented. The lowest effect concentration, 334 mg/kg, was exceeded by the majority of sediment fraction values reported here (cf Tables 1 and 8), and uniformly exceeded only those in sediment from site 1 (the reference site). It is therefore likely that effects on feeding would be present in situ throughout the entire stream segment under investigation. Maintenance of feeding rate at a low level even at very high food zinc concentrations indicated that the observed effects were related to food palatability and behaviour rather than to sublethal toxicity. Since sediment contamination appears, on the basis of present measurements, to persist throughout the year, the biological effects may also be expected to persist. Feeding rates in the dietary study were markedly lower than in the sediment exposure study. This may have been because the animals used had, prior to the experiment, been fed ad lib for some time on leaf material well-colonised with microfungi and may have become conditioned to this type of food.

SECTION 5 - CONCLUSIONS

Experimental evidence has shown that, while sediments derived from watercress farm discharges are not directly toxic, the levels of contamination present throughout the study area in October 1989 were sufficient to cause a substantial reduction in energy consumption. This reduction is likely to be a consequence of a behavioural response to contaminated particulate material, and may be exacerbated by a general unsuitability of sediment granulometry in terms of habitat structure and food item size, although this latter question remains to be tested. In contrast, sublethal biological responses would not be expected at the highest ambient water concentration measured in the present study; statistically significant effects on feeding rate in Gammarus were reported by Maltby et al (in press) at 0.5 mg/l, but not at 0.3 mg/l - some ten times higher than our maximum value. McCahon and Pascoe (1988) have, however, shown that juvenile Gammarus may be up to 250 times as sensitive to metals as are adults. The possibility remains that Gammarus might actively avoid zinc concentrations lower than those causing sublethal toxicity, and that winter dosing of zinc might contribute to population losses through drift. There is some experimental evidence that Gammarus will avoid dissolved zinc (Costa 1966, Abel and Green 1981), although there is a shortage of data relating to the concentrations measured in UK receiving streams.

A reduction in feeding rate of at least 25% might be expected in response to the levels of particulate contamination measured in the present study. This, in itself, would have a serious impact on population processes, and could lead to a reduction in fecundity and recruitment. There appears to be a fairly narrow margin between 'effect' and 'no-effect' concentrations, and this will tend to render receiving streams sensitive to relatively small perturbations.

The effects of zinc contamination of food can be expected to apply to all substrate and habitat types, since in less heavily-sedimented zones the available leaf material will still be contaminated by zinc - in the dietary zinc experiment, exposure to only 21 µg/l resulted in leaf zinc concentrations of 334 mg/kg, sufficient to significantly reduce feeding rate.

The proportion of total habitat occluded by fine sediments may be important for a number of reasons. Gee (1982) has noted that Gammarus move faster over fine sediments; this may have energetic costs and also increase the likelihood of animals entering the drift. Marchant (1981) reported that at high densities, Gammarus was more likely to enter the drift, and suggested that competition for space was the reason. Since, in the present study, fine sediments have been shown to have limited ability to support Gammarus, it is possible that a reduction in suitable habitat area limits the theoretical carrying capacity of the stream. Nilsson and Sjostrom (1977) also noted the importance of carrying capacity in controlling colonisation. A reduction in available substrate would adversely affect juvenile animals most. Adams et al (1987) showed that Gammarus distribution was related to substrate size and current velocity; small animals were found in substrates with smaller interstitial spaces, and such substrate has the highest likelihood of being subjected to gross sedimentation by virtue of its association with lower current velocities.

In summary, the investigation has shown that:

a) fine sediments derived from watercress farms are marginal habitats for Gammarus;

b) zinc contamination of sediments along the entire 3.5 km stretch of river investigated is sufficient to cause significant reductions in feeding rate;

c) the levels of leaf contamination likely to arise from ambient winter dissolved zinc concentrations are sufficient to cause significant reductions in feeding rate; leaf Zn concentrations which were associated with such reduction in laboratory studies were similar to concentrations determined in field samples.

d) the effects of zinc contamination of particulate material are most probably behavioural rather than toxic;

e) the persistence in sediments of zinc contamination at observed levels throughout the year would be sufficient to reduce, and possibly to eliminate, Gammarus populations.

SECTION 6 - RECOMMENDATIONS

Further attention should be given to the contribution of reduction in habitat area and suitability resulting from excessive sedimentation. The demonstration of behavioural responses in terms of feeding rate to particulate zinc and to in-situ sediments, does not preclude the possibility of behavioural responses in terms of avoidance. In principle, animals might enter the drift at lower levels of zinc than those affecting feeding; in practice, effects on feeding were observed at concentrations fairly close to 'background'.

In order to mitigate the probable in-situ effects of zinc contamination on feeding rate, both dissolved and particulate zinc inputs from watercress farms would need to be reduced. Further research would be required to determine, with confidence, the limit on concentration of a dissolved zinc needed to control particulate zinc concentrations to an acceptable level. The EQS value of 75 µg/l designed to protect sensitive aquatic life probably does not restrict contamination of Gammarus food material to a level which the species can tolerate without adverse effects.

Trapping of vegetation debris at the outflow from watercress farms would seem to be necessary to effect an increase in the area of suitable Gammarus habitat available in receiving streams.

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Table 1 - Sediment collection sites on the Pillhill Brook

Site	Distance downstream	Map reference
1	Parallel to farm-reference site	SU 3280 4389
2	Effluent channel	SU 3280 4382
3	750 metres	SU 3347 4374
4	2100 metres	SU 3459 4404
5	3200 metres	SU 3552 4417

Table 2 - Zinc concentrations in Pillhill Brook water samples (µg/l total Zn)

Site	1	2	3	4	5
Concentration	<0.003	0.027	0.008	<0.003	0.011

Table 3 - Zinc concentrations (mg/kg) in whole and fractionated Pilhill brook sediments: data from four pooled sediment cores per site

Site	Fraction				
	>250	125-250	<125	Leaf litter	Whole
1	162	142	124	121	145
2	415	359	316	278	367
3	379	358	309	330	365
4	495	370	302	853	451
5	372	439	351	559	399

Table 4 – Distribution of zinc between sediment fractions: percentage of total zinc in each fraction

Site	Fraction			
	>250	125-250	<125	Leaf litter
1	48	30	15	6
2	44	35	17	4
3	67	21	11	1
4	58	22	10	10
5	32	50	17	1

Table 5 - Sediment mass distribution by size fraction (percent of total sediment sample mass)

Site	Fraction			
	>250	125-250	<125	Leaf litter
1	43	30	18	8
2	39	37	19	5
3	65	21	13	<1
4	53	27	14	5
5	34	46	19	<1

Table 6 - Percent volatile solids content of sediment fractions ('organic' content estimated from weight loss on ignition at 450 °C) ND = not determined

Site	Fraction			
	>250	125-250	<125	Leaf litter
1	17.2	7.6	4.4	66
2	31.5	13.7	9.0	66
3	15.8	9.6	7.6	40
4	12.5	2.3	0.9	ND
5	9.3	8.5	3.5	ND

Table 7 - Overall max-min water quality data during exposure of *Gammarus* for trays holding sediment cores from five sites

Site	Temperature (°C)		DO (%asv)		pH	
	Max	Min	Max	Min	Max	Min
1	12.8	11.0	99	83	8.56	8.23
2	11.8	9.90	99	81	8.56	8.15
3	12.9	11.70	99	87	8.53	8.11
4	12.7	9.90	99	82	8.57	8.21
5	12.5	11.10	100	85	8.55	8.22

Table 8 - Numbers of Gammarus which died, moulted or escaped during eight weeks exposure to sediments from five sites

Site	Original number	Final number	Dead	Moulted	Escaped
1	40	35	1	4	2
2	40	35	2	3	1
3	40	37	3	0	1
4	40	37	2	1	1
5	40	35	3	2	2
Total	200	179	11	10	7

Table 9 - Percentage of animals in contact with sediment on each of 19 observation dates over a period of eight weeks. Observations are for fed animals only

Observation	Site					
	1	2	3	4	5	Mean
1	90	55	66	63	78	70
2	83	50	66	60	100	72
3	77	88	73	82	60	76
4	90	77	64	60	92	77
5	100	100	64	90	75	86
6	100	100	82	100	100	96
7	100	100	64	91	83	88
8	92	100	55	82	82	82
9	77	55	50	91	75	70
10	70	77	73	80	55	71
11	88	88	70	89	63	80
12	100	77	77	82	78	83
13	55	100	77	50	45	65
14	100	90	90	80	73	87
15	90	80	80	70	73	79
16	90	80	70	63	64	73
17	70	88	80	60	73	84
18	100	77	90	89	63	84
19	90	80	70	80	80	80
Mean	84	82	71	77	74	79

Table 10 - Mean feeding rates (J/mg dry wt/d) of *Gammarus* exposed to Pilhill Brook sediment cores for four and eight weeks, with and without food supplement

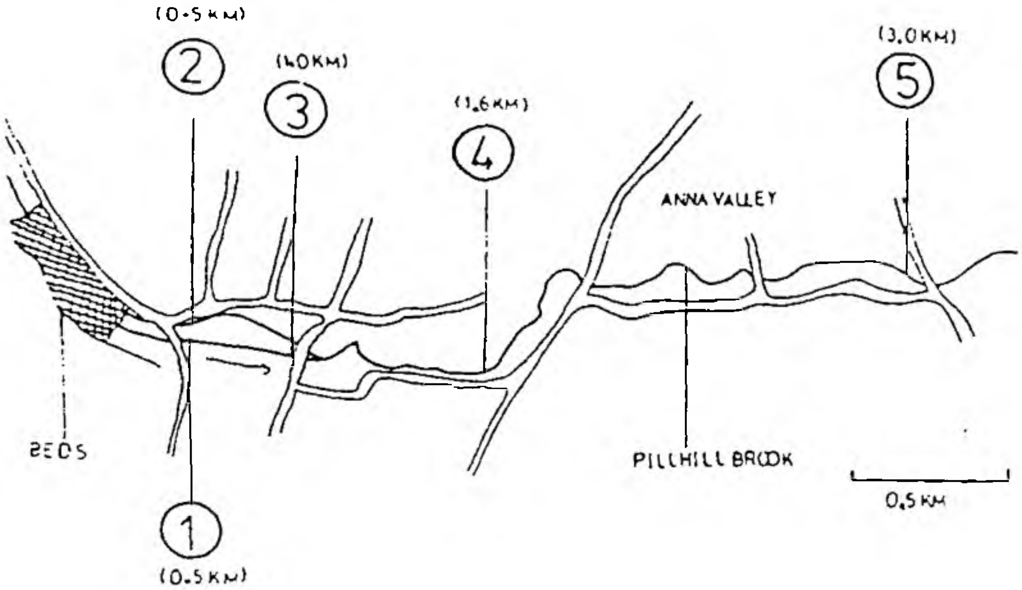
Site	Four weeks		Eight weeks	
	Fed	Unfed	Fed	Unfed
1	6.29	10.23	6.66	6.20
2	6.91	11.43	6.66	6.45
3	10.22	7.84	6.63	6.83
4	5.28	9.33	5.39	8.30
5	7.67	7.78	7.27	7.04

Table 11 - Mean dry weights (mg) of *Gammarus* at four and eight weeks immediately following feeding rate measurements

Site	Four weeks		Eight weeks	
	Fed	Unfed	Fed	Unfed
1	8.00	7.35	9.71	6.35
2	8.24	6.82	10.91	7.64
3	8.77	7.50	9.34	7.04
4	8.62	8.34	9.35	7.53
5	9.48	8.49	8.25	6.99

Table 12 - Feeding rates (J/mg/d) of *Gammarus* exposed to alder leaf discs experimentally contaminated with zinc

Concentration in leaf (mg/kg)	Feeding rate (J/mg/d)	Dry weight (mg)
230	2.74	6.33
334	2.33	7.43
391	2.07	7.18
1050	1.85	6.61
4160	1.41	6.82



Abbots Ann Cress Farm.

1 (SU 328 438)
2 (SU 328 439)
3 (SU 332 438)
4 (SU 336 438)
5 (SU 351 440)

(KM) DENOTES DISTANCE FROM CRESS BEDS.

Figure 1 Sediment collection sites in Pilhill Brook downstream of Abbots Ann watercress farm

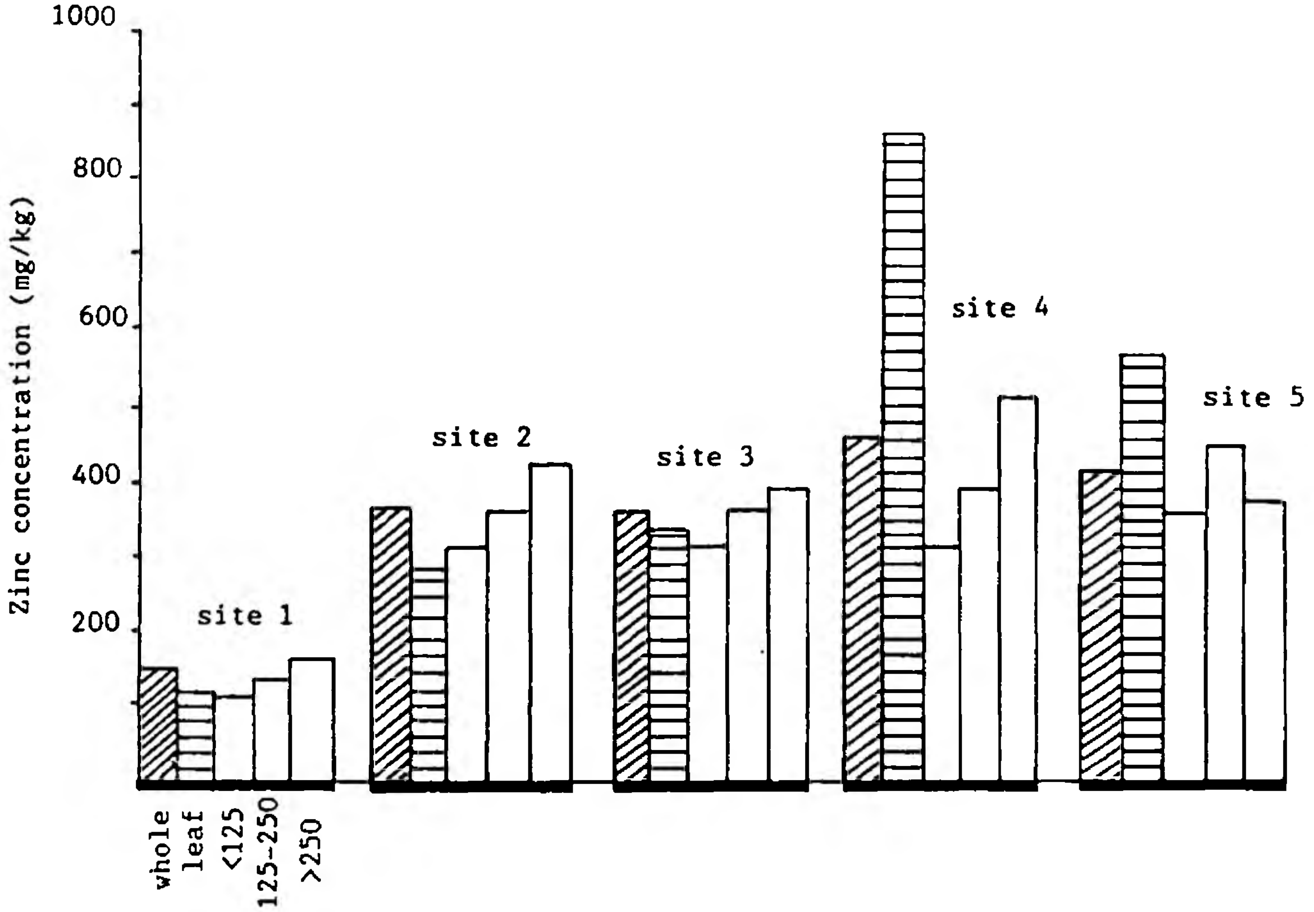


Figure 2 Concentrations (mg/kg) of zinc in fractions of sediment from five sites on Pilhill Brook

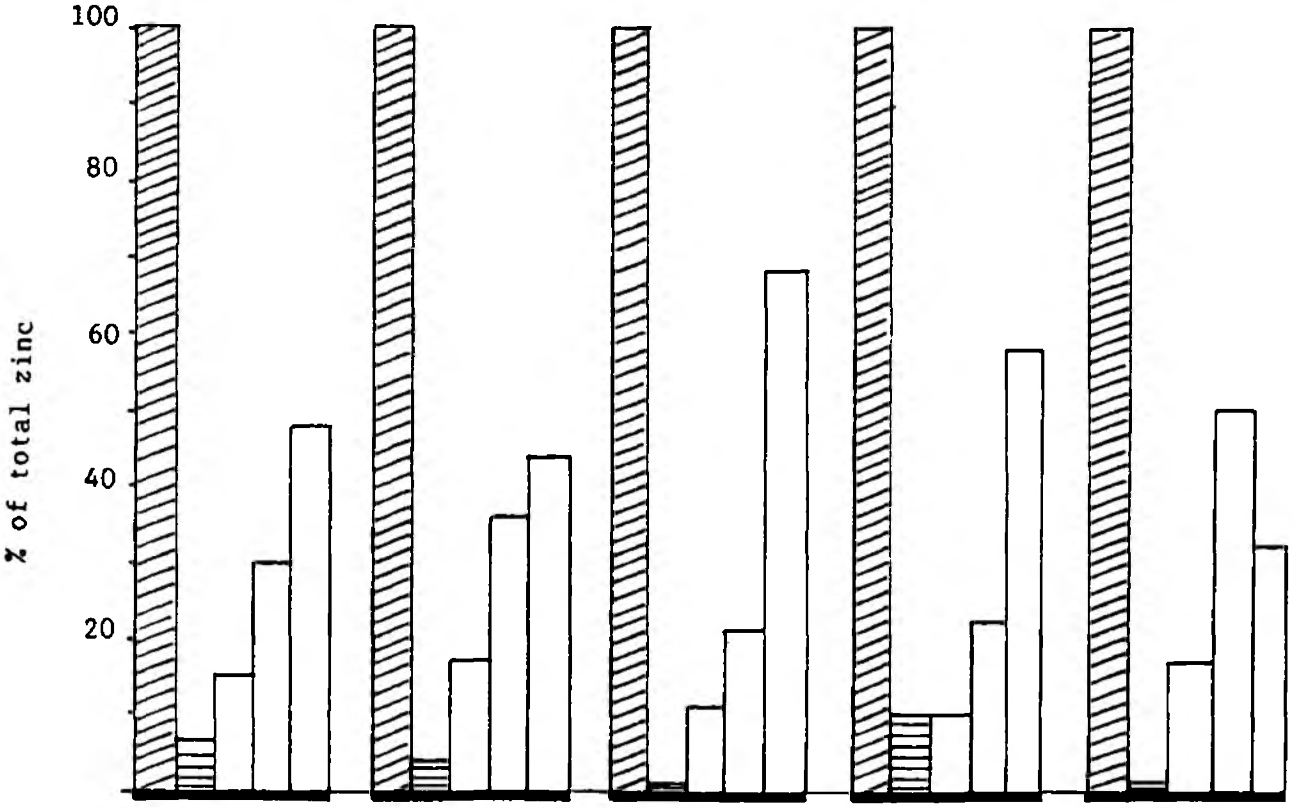


Figure 3 Percentage distribution of zinc in sediment fractions

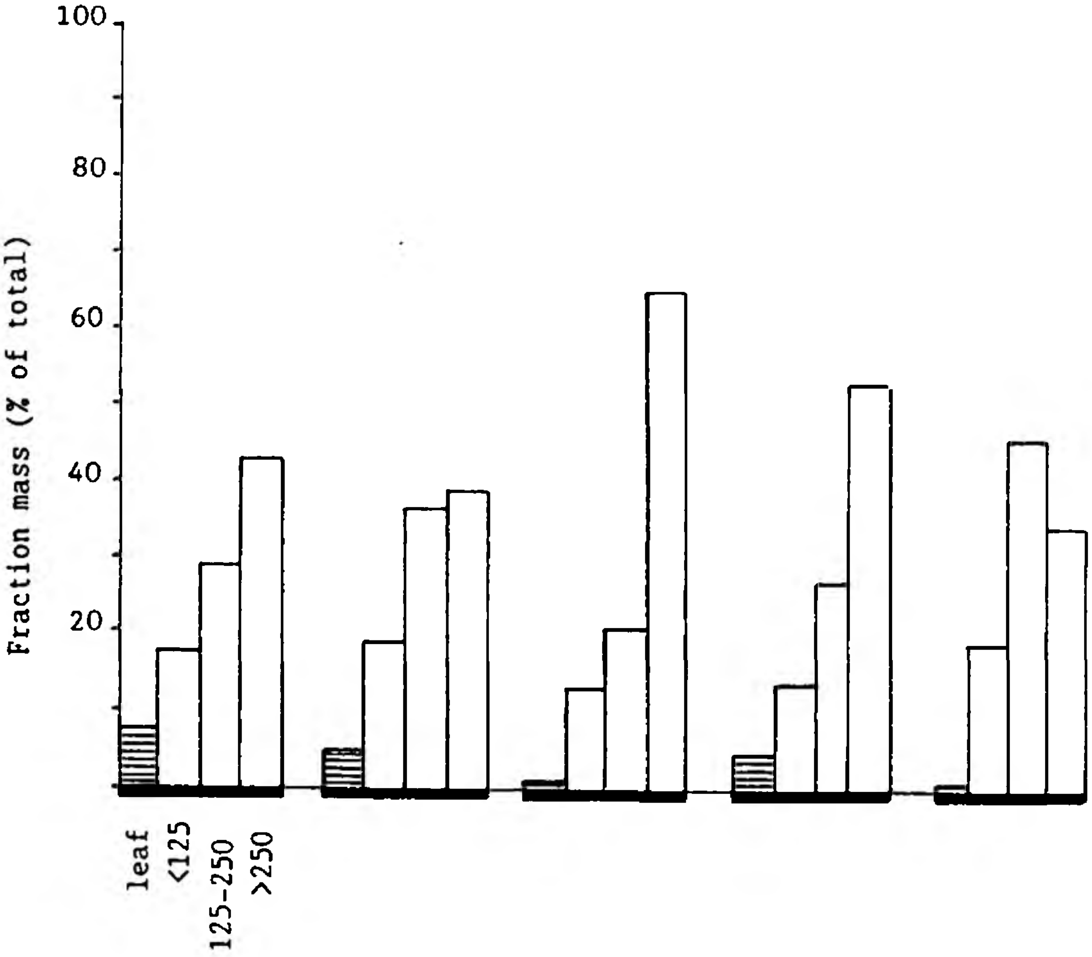


Figure 4 Distribution of mass in sediment fractions from Pilhill Brook sediments

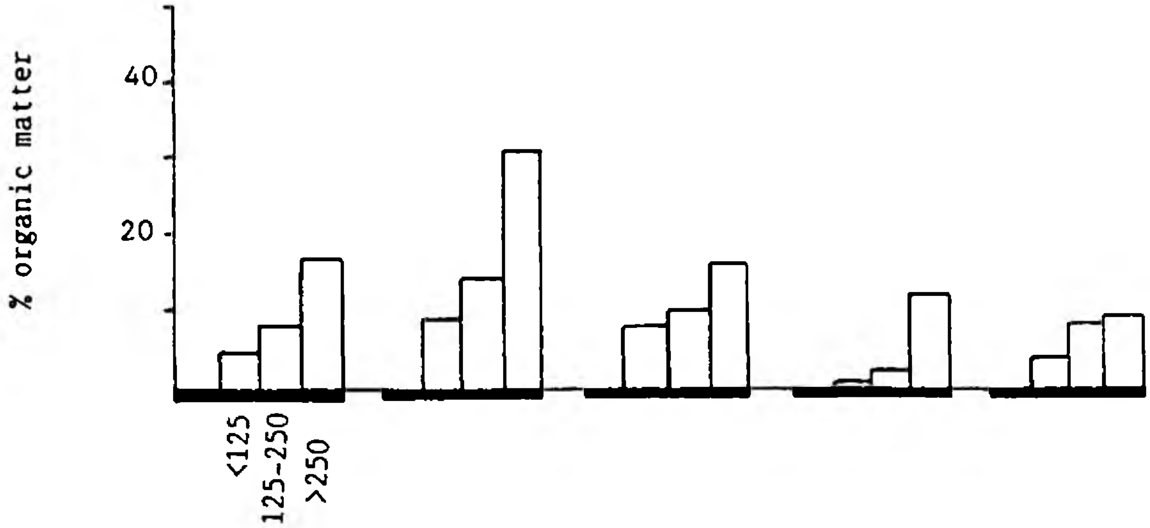


Figure 5 Percent volatile solids ('organic' fraction) in different fractions of Pilhill Brook sediments from five sites

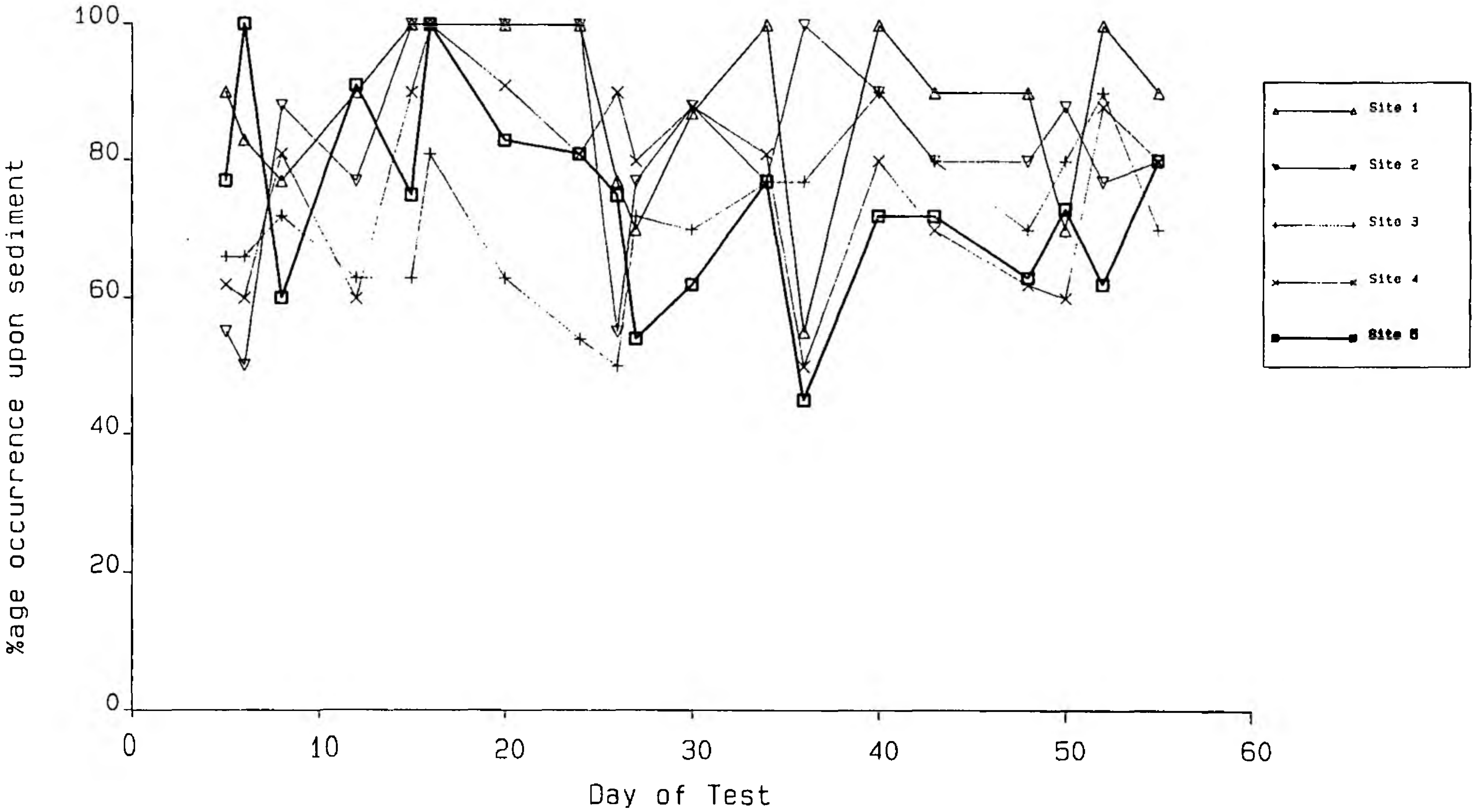


Figure 6 Percentage occurrence of Gammarus in contact with sediment during 8 weeks' exposure to Pilhill Brook sediment samples

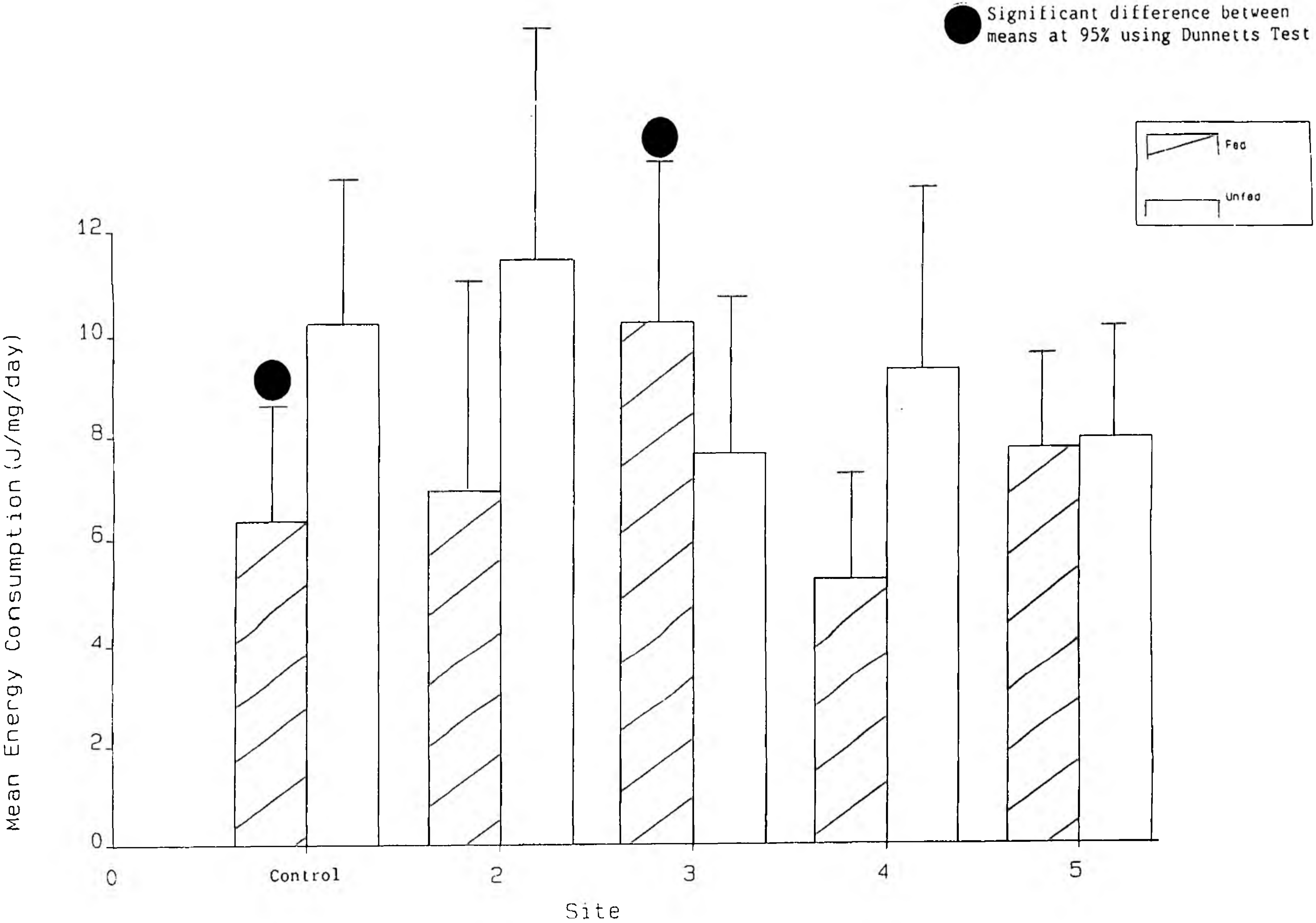


Figure 7 Mean energy consumption rate (J/mg/d) of Gammarus exposed to Pilhill Brook sediments for 4 weeks. Error bars are 95% confidence intervals.

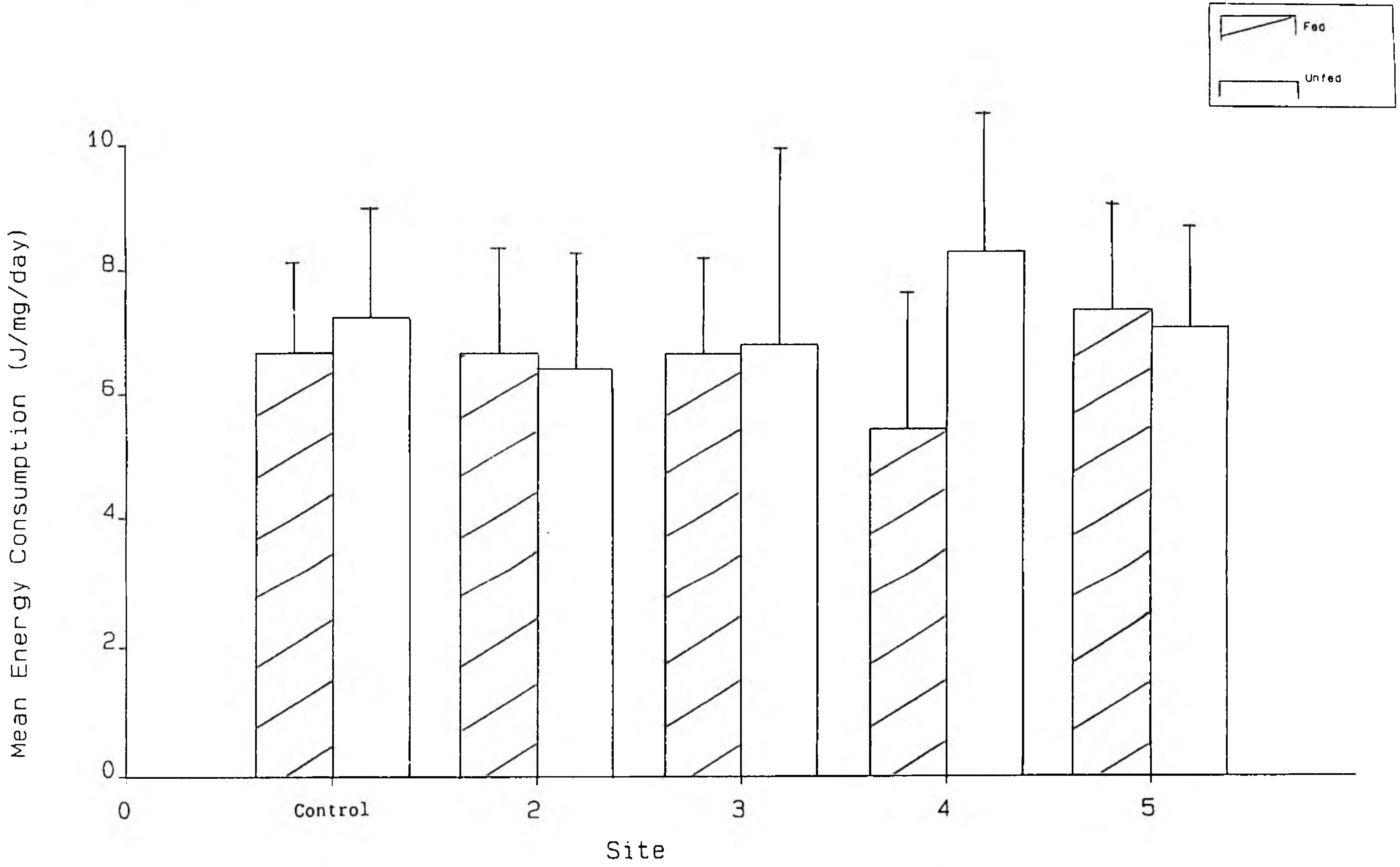


Figure 8 Mean energy consumption rate (J/mg/d) of Gammarus exposed to Pilhill Brook sediments for 8 weeks. Error bars are 95% confidence intervals.

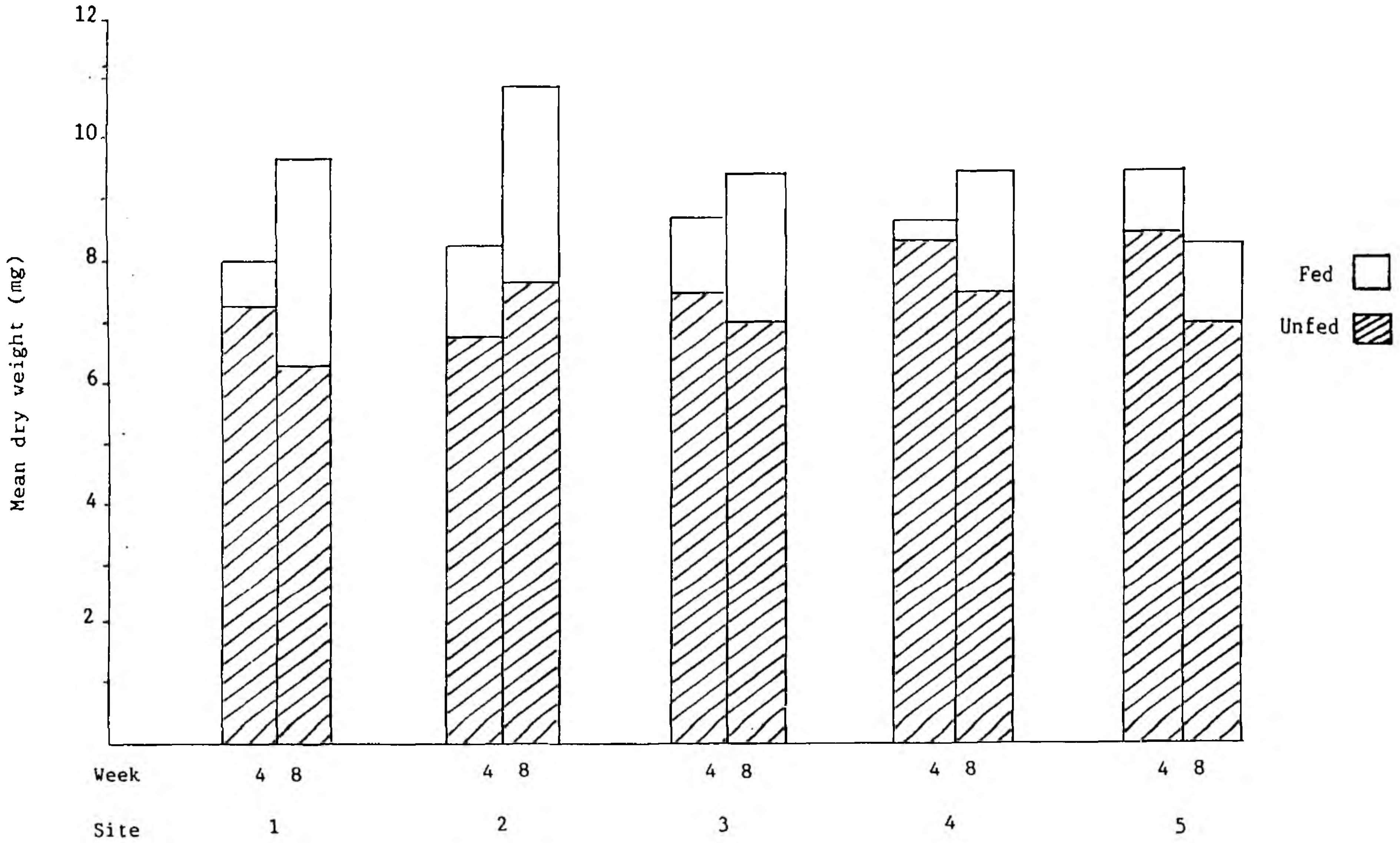


Figure 9 Mean dry weight of <u>Gammarus</u> following four and eight weeks exposure to stream sediments, with and without supplementary food

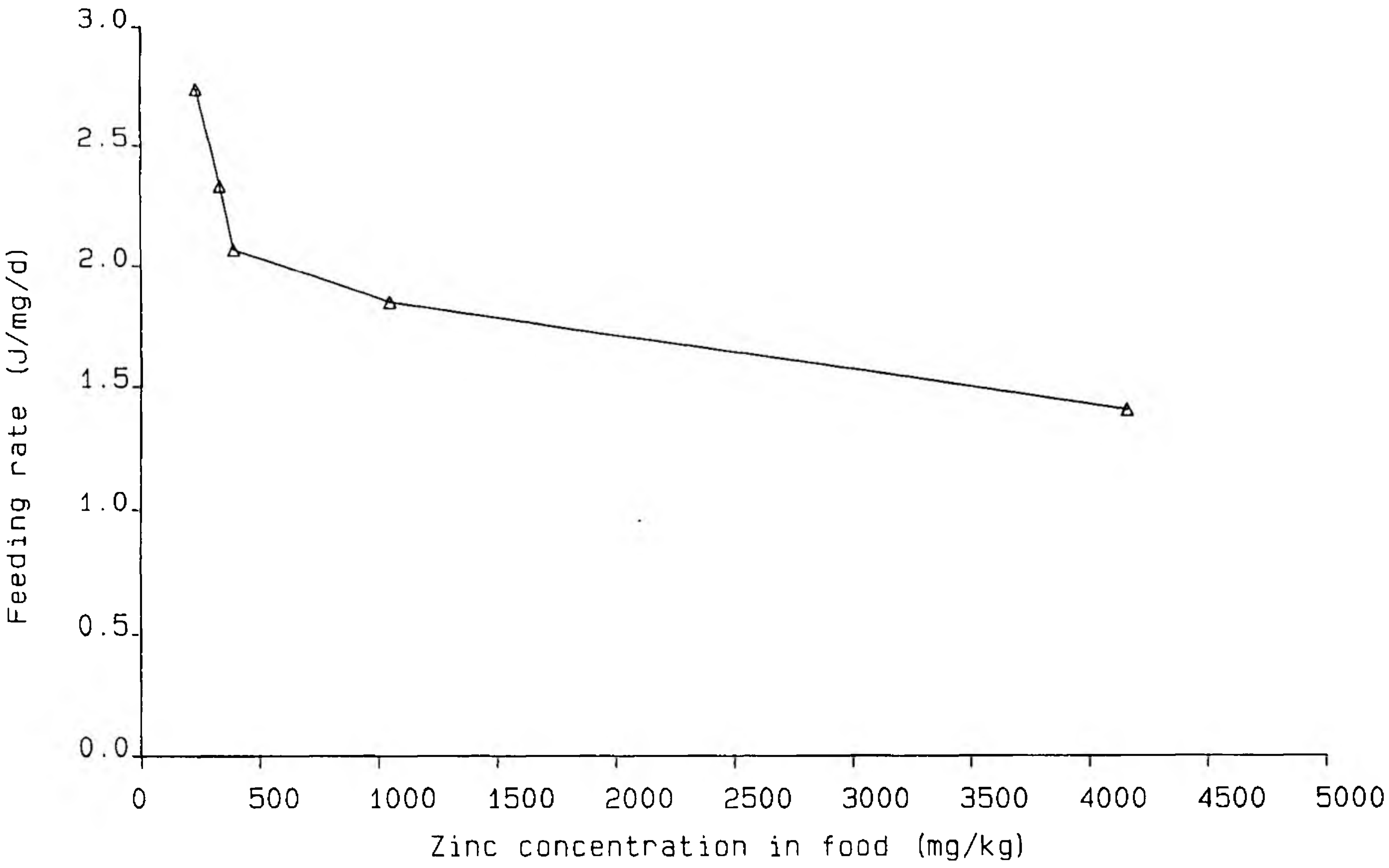


Figure 10 Mean energy consumption rate (J/mg/d) of *Gammarus* fed for 10 days on zinc-contaminated leaf material

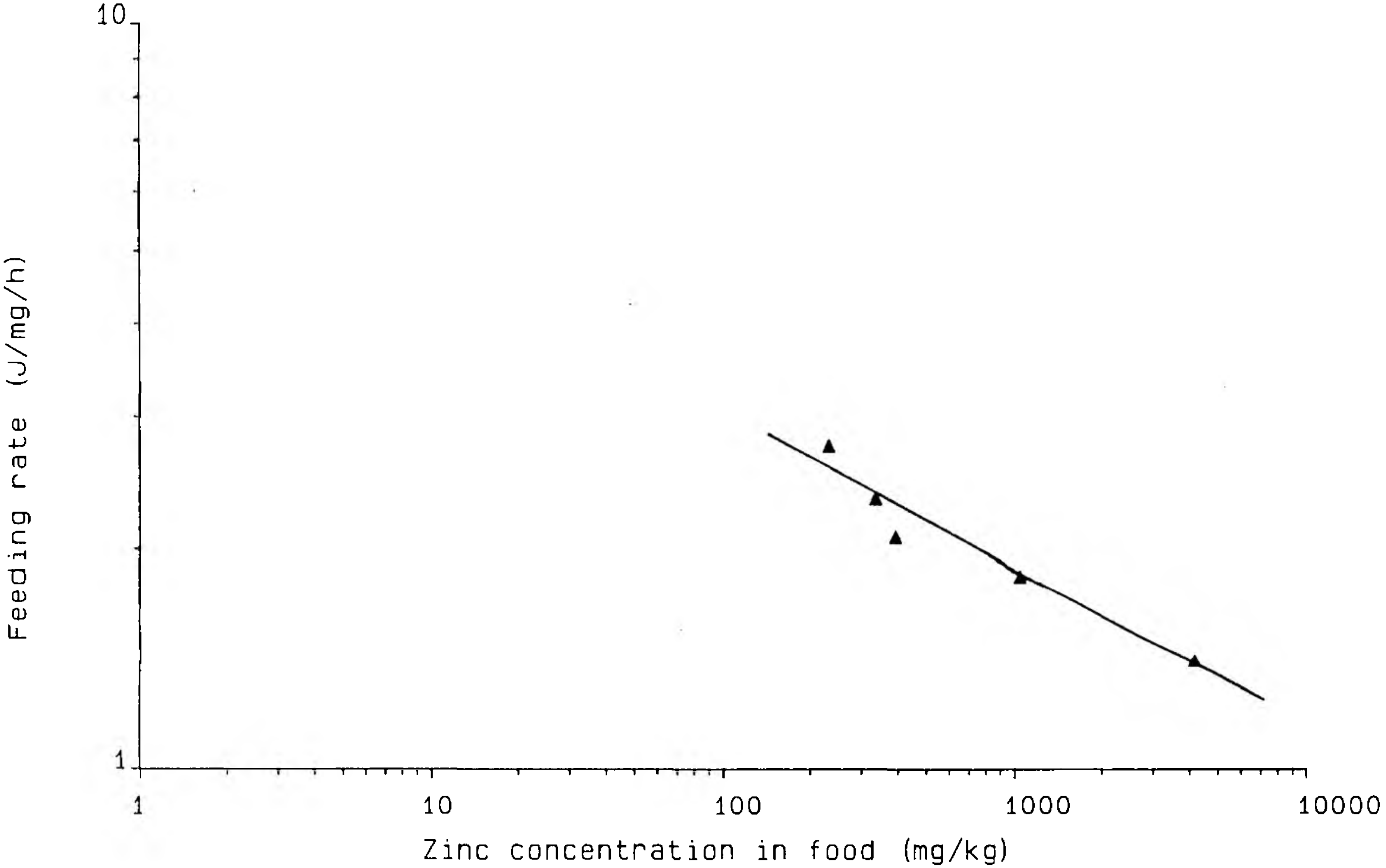


Figure 11 Mean energy consumption rate (J/mg/d) of Gammarus fed for 10 days on zinc-contaminated leaf material: logarithmic scales