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Potential Impact of Watercress Farm Discharges on the Freshwater Amphipod, *Gammarus pulex* L.

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The watercress industry in the United Kingdom uses zinc salts to control root infections and, as a consequence, discharges dissolved and particulate zinc to receiving waters throughout much of the year. National Rivers Authority (NRA) personnel have noted the selective elimination of the freshwater amphipod *Gammarus pulex* L. from many receiving streams for distances of several kilometers from the point of farm water discharge (Dr. J. Wharfe, NRA Southern Region, pers. comm.). *G. pulex* is an abundant and functionally important detritivore and fish food item in many British streams (Willoughby and Sutcliffe 1976; Welton 1979), and its disappearance is therefore a matter of some concern.

The objectives of this study were to assess the level of zinc contamination in water and sediment above and below a watercress farm discharge, and to expose laboratory populations of *G. pulex* to zinc-contaminated sediment or food and monitor the effects of exposure on survival and feeding rate.

MATERIALS AND METHODS

A watercress farm at Abbots Ann in Hampshire (NGR SU 378438) was selected for the study, after consultation with NRA personnel. Effluent from this farm is discharged into an artificial channel which joins Pilhill Brook approximately 1 km below the farm. Five sites were selected between the farm and the confluence of Pilhill Brook with the River Anton. Site 1, on Pilhill Brook, adjacent to the farm, was above the farm discharge, although operations from other farms located upstream had led to a mixture of typical chalk stream and finer particled substrate patches. Site 2, immediately outside the farm boundary in the artificial channel, was expected to reflect the maximum impact of

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the discharge. The remaining three sites were located in Pilhill Brook, below the confluence with the artificial channel, at distances of 750 m, 2100 m and 3200 m, respectively, from the farm. The bed of the watercourse at sites 2 to 5 was almost completely covered by fine sediment particles produced by the watercress farm. NRA personnel reported the presence of a *G. pulex* population at site 1 and the absence of *G. pulex* from sites 2 to 5.

Forty-four sediment cores were collected from each site on 27 September 1989, using pipe corers 8 cm long, with an internal diameter of 6.5 cm. Cores were chosen at random points on the stream bed at sites 2 to 5, but only at random points within the finer substrate patches at site 1, in order to maximize the physical similarity between groups. Four of the cores from each site were pooled and all identifiable leaf material removed, dried at 80°C for 48 hours and weighed. The remaining material from these four cores was wet-sieved through 250 µm and 125 µm meshes. The three fractions were dried and weighed in the same manner as the leaf material. All fractions, including leaf material, were digested in nitric acid and analyzed for zinc (Standing Committee of Analysts 1986).

A single 100-ml water sample was collected at each site and preserved with nitric acid for subsequent determination of total zinc concentration by inductively-coupled plasma atomic emission spectrometry (Thompson and Walsh 1983).

The remaining forty cores from each site were placed in a 12°C constant temperature room in randomly located trays filled with aerated groundwater to a depth of 5 cm above the sediment surface. Nylon-mesh collars (1-mm mesh size) were fitted to the upper end of each core to prevent the escape of the test animals and to allow the circulation of water over the surface of the core. One adult male *G. pulex* was then placed on each core. These animals were obtained from the River Teme (NGR SO 548682) and had been acclimatized to test conditions for two weeks (groundwater: pH 7.9-8.1, hardness c. 250 mg/l, temperature 12-13°C). Half of the animals on cores from each site were exposed for four weeks and half for eight weeks, and within each of these groups approximately half were unfed and half provided with supplementary alder leaf (*Alnus glutinosa* L.) food material. The test animals were monitored daily and any mortalities recorded. Temperature, pH and dissolved oxygen were monitored daily in each tray, and maintained between 10-13°C, 8.11-8.57, and 81%-100% saturation, respectively. After four or eight weeks of exposure to sediments the feeding rate of the *G. pulex* was measured over a six-day period in uncontaminated groundwater using the method described by Naylor *et al.* (1989).

The effect of dietary zinc on the feeding rate of *G. pulex* was assessed by immersing 17-mm diameter alder leaf discs in solutions of 0, 21, 135, 900 or 6000 µg Zn/l (as analytical grade ZnCl). The leaf discs were removed from their respective solutions after 48 hours and dried at 80°C for 2 days. Samples of approximately 20 mg dry weight of leaf disc per treatment were analyzed for zinc as described above. The zinc-contaminated leaf discs were then used in a feeding rate experiment with *G. pulex* obtained from the same source and kept under the same conditions as those used in the sediment exposure experiment. The method of Naylor *et al* (1989) was again used, but the duration of the test was increased from 6 to 10 days and each animal was provided with six, rather than four, leaf discs.

RESULTS AND DISCUSSION

Total zinc concentrations in stream water samples were below the detection limit of 0.003 mg Zn/l at sites 1 and 4, and 0.027, 0.008 and 0.011 mg Zn/l at sites 2, 3 and 5, respectively. Although concentrations were highest immediately below the watercress farm, they did not decline steadily with distance. Maltby *et al* (1990) found that the respiration and food assimilation rates of *G. pulex* were adversely affected at 0.5 mg Zn/l but not at 0.3 mg Zn/l. This suggests that the levels of zinc found in water were well below that capable of causing mortality, or sublethal effects on adult *G. pulex*, although McCahon and Pascoe (1988) have shown that juvenile *G. pulex* may be considerably more sensitive than adults to heavy metal contamination. In addition, only one spot water sample was taken at each site in the present study and there have been reports of higher levels of zinc below some watercress farms (Dr. H. Casey, Institute of Freshwater Ecology, pers. comm.).

Zinc concentrations were higher in the sediment fractions of samples from sites 2 to 5 than from site 1 (Table 1). 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.

Table 1. Zinc concentrations (µg/g) in whole and fractioned sediments: data from four pooled sediment cores per site

Site	Fraction (µm)				
	>250	125-250	<125	Leaf litter	Whole sediment
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

The sediment cores collected from the field were not acutely toxic to *G. pulex* over either a four- or eight-week period. No more than three out of forty animals died in any of the sediment exposure treatment groups. *G. pulex* feeding rates were also similar between the reference and test sites (Table 2, Dunnett's test $P>0.05$) which suggests that if the sediments were sublethally toxic to *G. pulex*, any effects on feeding rate disappeared rapidly after removal to uncontaminated conditions. However, unfed animals weighed significantly less than fed animals at the end of both the four week and eight week experiments (ANOVA $F=5.32$, $P=0.024$ and $F=20.58$, $P<0.001$, respectively). Fed animals had a mean dry weight of 8.63 mg at the end of the four week experiment, while their unfed counterparts had a mean dry weight of 7.70 mg. Fed and unfed animals had a mean weight of 9.52 mg and 7.09 mg, respectively, at the end of the eight week experiment. *G. pulex* provided with supplementary food, therefore, tended to gain weight, while those that had to rely on food present in the sediment cores tended to lose weight during the experiment. These results suggest that the sediment characteristics at all of the sites, including the fine sediment patches sampled at the reference site, were sub-optimal for *G. pulex* and that insufficient food was available for normal feeding requirements.

An approximately logarithmic decline in *G. pulex* feeding rate occurred with increasing zinc concentration in food (Table 3). *G. pulex* in all of the contaminated food treatment groups fed at a significantly lower rate than those in the control group (ANOVA $F=4.48$, $P<0.001$; Dunnett's test $q>1.93$,

Table 2. Feeding rates of *G. pulex* exposed to sediment cores for 4 or 8 weeks.

	Number of weeks exposure			
	Four		Eight	
Site	Fed	Unfed	Fed	Unfed
1	0.29, 0.15, (10)	0.47, 0.14, (7)	0.31, 0.09, (9)	0.33, 0.08, (6)
2	0.32, 0.23, (12)	0.53, 0.21, (6)	0.31, 0.12, (10)	0.30, 0.11, (8)
3	0.47, 0.11, (12)	0.36, 0.17, (8)	0.31, 0.10, (9)	0.32, 0.08, (3)
4	0.25, 0.15, (12)	0.43, 0.18, (7)	0.25, 0.15, (10)	0.39, 0.11, (6)
5	0.36, 0.14, (11)	0.36, 0.09, (7)	0.34, 0.13, (10)	0.33, 0.08, (6)

Values are mean feeding rate (mg dry weight of leaf material per mg dry weight of *G. pulex* per day) and standard deviation, with sample size in parentheses.

Table 3. Feeding rates of *G. pulex* exposed to zinc contaminated food for 10 days.

Concentration of zinc in food material (µg/g)	Feeding rate
230 (control)	0.127, 0.045, (20)
334	0.108, 0.058, (20)
391	0.096, 0.044, (19)
1050	0.086, 0.040, (20)
4160	0.065, 0.047, (20)

Values are mean feeding rate (mg dry weight of leaf material per mg dry weight of *G. pulex* per day) and standard deviation, with sample size in parentheses.

$P<0.05$). Levels of zinc in the experimentally contaminated leaf material were similar to those found in leaf material collected from sites impacted by the watercress farm effluent and it is therefore likely that effects on feeding would be present *in situ* throughout the entire stream segment below the farm.

This investigation has shown that fine sediments derived from watercress beds are marginal habitats for *G. pulex* and that zinc contamination of food material below watercress farms is sufficient to cause a reduction in feeding rate even when the concentration of zinc in the water column is low. Fine sediments are not a suitable habitat for this species (Gee 1982; Adams *et al.* 1987) and it is, therefore, unsurprising that populations have been eliminated where fine sediments predominate. However, the results from this study suggest that *G. pulex* is unlikely to re-establish itself if stream bed sedimentation alone is ameliorated. The accumulation of zinc in food material from only low levels in water must also be addressed.

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