Fine sediment effects on feeding and growth in the invertebrate grazers Potamopyrgus antipodarum (Gastropoda, Hydrobiidae) and Deleatidium sp. (Ephemeroptera, Leptophlebiidae)

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Abstract
The influence of fine sediment (<63 μm diameter) upon the assimilation rates of the small Potamopyrgus antipodarum, and the much larger Deleatidium sp. were determined by allowing individuals to feed upon 14C-radio-labelled periphyton which had been contaminated with varying quantities of sediment (sediment:food ratios of 0:1, 1:1, 5:1, 10:1, 50:1, 100:1 dry weight). For both grazers, the assimilation rate falls in direct proportion to the sediment fraction in the (sediment:food) mixture. In a second experiment, the growth of P. antipodarum was monitored over 3 months with fed organic matter that had been contaminated with fine sediment at ratios of 0:1, 1:1, 5:1, 10:1, 50:1, 100:1 dry weight) and lowest in the treatment with no sediment added. Growth rates were significantly lower, and mortality high, at sediment:food contamination ratios above 50:1. The reasons for the contrast between the ratios in the short-term and the long-term experiments are unknown at present, but the fact that small growth was greatest at intermediate levels of sediment contamination might indicate that they derive trace nutrients from inorganic sediment.

Introduction
Increased sediment loads in aquatic ecosystems often follow land-use change and human activities such as agriculture, forestry-cultivation, mining and urbanisation. For example, during a 3-year period of quarterly monitoring at the Whakatata kilohectare Agricultural Research Station (west of Hamilton, North Island, New Zealand), epilithic DW:AW ratios have varied between 3.1 and 10.1 (excluding 6.1 in only four of 27 samples) in intact or regenerating native forest reaches, whilst in otherwise aquatic pasture streams they have ranged between 3.1 and 82.1, exceeding 6.1 in 12 of 48 samples. Similarly, Davies-Colley et al. (1992) report that epilithic DW:AW ratios ranged between 3:1 and 10:1 above the points at which several gold mines discharged their sediment-rich (pyritic) waste-water, but between 10:1 and 20:1 below the discharges, and Quinn et al. (1992) report that macro-invertebrates (dominated by insecta) trophic diversity and abundance were both substantially lower at the downstream sites.

High sediment loads, particularly the deposition of fine sediments, have adverse effects on fish through clogging of gill and spawning grounds (Sear, 1993), and reducing feeding success in turbid streams (Rowe & Dean, 1998). Similarly, increased sediment loads may reduce the abundance and diversity of invertebrates by smothering interstitial habitat and reducing periphytic
abundance or quality (Lloyd et al., 1987; Newcombe & MacDonald, 1991; Ryan, 1991; Wood & Armitage, 1997).

Fine substrates have been found to be unsuitable habitats for most New Zealand aquatic insects (Quinn & Hickey, 1990; Jowett et al., 1991; Death, 2000). Some New Zealand invertebrate species, notably Dacaulus spp. (Ephemeroptera: Lepidopteridae) and Protonemurus spp. (Trichoptera: Cono- clydia) show preferences for 'clean' rather than silty periphyton (Ryan, 1991) and, in colonization trials, Ryder (1989) showed that the occurrence of fine sediment in the algal matrix reduced invertebrate densities by about 30%. Consequently, changes in the pattern of sediment deposition as a result of landuse change may modify the impact that invertebrate grazers have upon their periphytic prey. The Hydrobiont small Protonemurus sp. is often dominant in the macroinvertebrate communities in low-order pasture streams throughout New Zealand, yet is rare in otherwise similar polluted catchments in which the 'sensitive' Ephemeroptera, Plecoptera and Trichoptera taxa usually dominate (Quinn & Hickey, 1990). Of these latter taxa, the Lepidopteridae notably Dacaulus spp., is often the most abundant species, and this has been shown to be unable to separate food from silt prior to ingestion and to be less abundant on sediment-enriched tiles than on sediment-poor ones (Ryder, 1989; Ryan, 1991). In contrast, P. anoplodactylus was more abundant on the impacted tiles (Ryder, 1989) and has been shown to take small (silt) sediment particles into the buccal cavity, scrape the encrusting organic matter off and finally 'spit' the sediment particles out (Lopez & Kofoid, 1989).

These various lines of evidence suggest that P. anoplodactylus may be more tolerant of sediment contamination in its food than Dacaulus spp. are. We seek to determine whether this is so by: (a) determining the manner in which the assimilation rate is related to sediment contamination levels in the two species; and (b) determining the long-term growth rates of P. anoplodactylus at a variety of sediment-food ratios.

Methods

Smalls were collected from a reach draining pastoral land in the Whiteman's Valley Research Centre, west of Hamilton, New Zealand in December 1999, and the mayflies were collected from the same site in late January 2000. On each occasion, the invertebrates were brought back to the laboratory and held in aerated water at a 20 °C (near ambient for the level pasture streams at the time of year) controlled temperature room under permanent darkness until required. The invertebrates were provided with a combination of epithelium-encapsulated stones together with fresh (largely waterweed, Nasturtium officinale) and decaying vegetation & effluents and remains of algal or other material such as bankside grasses that were taken from the reach, where the invertebrates were collected, as food sources.

A stock of sediment was obtained from a landslip site within the Whakatane Research Centre prior to the experiment. This was washed through a series of sieves. Sediment which passed through a 63 μm sieve was retained and concentrated to a slurry by evaporation. The sediment content (measured as dry weight, DW) of this slurry was then determined by drying a known volume at 100 °C overnight in a preweighed dish.

Assimilation experiment

Individual smalls (Protonemurus anoplodactylus) and mayflies (Dacaulus sp.) were fed excess 14C radiolabelled periphyton contaminated with varying quantities of fine sediment and the quantity of periphyton assimilated over a period of 12-14 h was measured. Periphyton was harvested from 20 cm2, rectangular mats in December 1999. These had been growing under direct sunlight in artificial stream channels at the Whakatane Research Centre for 2 months. The harvested periphyton was maintained as a slurry in gently stirred, aerated vessels until required. Sediment originally present within the harvestable periphyton quickly settled to the bottom of the aquarium. Sub-samples of the slurry were drawn from mid-depths required. The taxonomic composition of the periphyton was not examined and it was dominated by unicells. In an earlier, summerize experiment in these same channels, Quinn et al. (1997) found that unicellular diatoms dominated the unseeded channels (77% by cell numbers), filamentous diatoms made up a further 15%, flagellates made up 4%, and 2% of other unicellular algae were present in small quantities in our stock slurry, but would have been rare in the realised food preparations because they were not readily drawn into pipettes used during the various sub-sampling procedures described below.

The concentration of the periphyton slurry was determined by filtering two replicate 1 ml samples onto
pre-weighted glass fibre filters and drying them at 100 °C overnight. The ash-free dry weight/dry weight (AFDW/DW) ratio of the periphyton was also established on one occasion by subsequent combustion at 400 °C overnight and reweighing of the filters.

Periphyton for each grazing trial was prepared as follows: A subsample was taken from the periphyton slurry and incubated at 20 °C with 14C bicarbonate (5-6 μCi/L) under fluorescent light (<150 μW m⁻²) and held in suspension using a magnetic stirrer for approximately 12 h. Excess free label was always present at the end of incubation. After incubation, the labelled suspension was drawn off and the periphyton centrifuged at 1400 rpm for 3 min and then resuspended in deionised water. This procedure was repeated three times. In order to minimise the quantity of unincorporated radio-labelled associated with the periphyton (preliminary trials indicated that this reduced the activity in the suspension to <5% of that after the first spin (i.e. of the levels at the end of the incubation period, with little further reduction thereafter). Finally, four replicate samples of 2 ml were drawn from this "clean" periphyton suspension. Two were used to provide continuous measurements of the periphyton concentration (filtered and dried), and two were used to derive estimates of the radiocesium in the periphyton. These latter samples were placed in scintillation vials together with scintillant cocktail (Organophase RS43) and counted in a Wallace 1409 (liquid) scintillation counter.

An appropriate quantity of the "clean" suspension of labelled periphyton was then pipetted into a 120 ml alkali-lime, screw-top container in order to yield a food density of (approximately) 60 μg DW m⁻². Appropriate quantities of the sediment slurry were also added in a similar manner in order to yield six different sediment-food ratios. These were 0.5, 1.1, 3.5, 10.1, 50.1 and 100.1 (by DW). Twelve replicates of each treatment were established. The containers were then topped up with filtered stream water and stirred. After 30 minutes, the periphyton suspension had settled, a single grazier of known size (P antipodarum; 5-9 mm shell height; Delphinium: 4-7 mm body length) was added to each container. Two of these individuals were dead (killed with scalding water) and operated as controls. Each container was sealed using a fine plastic mesh and ventilated using a whisker blower (Hickey & Vickers, 1992) and the grazers incubated at 20 °C under lower intensity (<59 μW m⁻²) fluorescent light for 12-14 h. At the end of this period each grazier was transferred to a clean container and left to empty its gut for 1-3 h. Grazers were subsequently removed and thoroughly cleared of any adherent algae by rinsing with deionised water (Delphinium sp) or scrubbing with a toothbrush and rinsing with deionised water (P. antipodarum). The individuals were subsequently killed by placing in isocapnic water. Each dead individual was placed in a scintillation vial with 0.5 ml of NCS-H tissue solvent and left for a minimum of 24 h. The small size of P. antipodarum makes it difficult to remove all of the soft tissue parts from the shell reliably, and so the snails were simply crushed in their shells before the solvent was added. After digestion, the radiocesium in each grazier was counted using the scintillation counter.

Passive uptake of bicarbonate-14C by the snail shell was discounted by subtracting the mean 14C count of the two control (dead) snails in each treatment from the counts for each live snail in the treatment. Thus, for both P. antipodarum and Delphinium sp. individual assimilation rates were calculated as:

\[ \text{A} = \frac{24 \text{ h DPM}_{\text{grazer}} - \text{DPM}_{\text{control}}}{H - \text{DPM}_{\text{periphyton}}} \]

in which A is the grazer's assimilation rate (g periphyton AFW d⁻¹), DPMgrazer denotes the decay counts in the grazers (decays per minute), and DPMcontrol denotes the average of the counts in the two dead (control) grazers from this food density treatment. DPMperiphyton denotes the decay counts per minute per g AFW of periphyton, and H denotes the density of the experiment (g).

Snail shell heights were measured with digital calipers (0.01 mm) prior to the beginning of an experiment. Mortal body lengths were measured after death. Shell lengths were converted to weights using established weight-length relationships (Tows et al., 1994) and the individual assimilation rates were expressed as biomass-specific rates. In the case of P. antipodarum, this is based upon the CaCO3-free weight (assuming this to represent 78% of the whole animal DW; Teves et al., 1993) of the snail to allow comparison with Delphinium sp.

Growth experiment

Individual P. antipodarum were reared on a diet consisting of excess organic matter contaminated to varying degrees with fine sediment and their growth was monitored over a period of about 3 months. Commercial chicken feed (Hatfley Farms Stock Feed
A "py-Lav") was used as the food source in place of peptone as its supply and quality could more easily be maintained throughout the experiment. The food characteristics are as follows: minimum crude protein 4.5%, maximum crude fat 3.5%, maximum crude fiber 5%, and minerals 0.6% (by weight). Its principal ingredients are maize, barley, wheat, legumes and milk products, brewer's yeast, vitamins, and minerals and are also included.

Seven treatments (ratio of sediment DW to food DW) were established. Three ratios were 0.1, 1-1, 5-1, 10-1, 50-1, 100-1, and 500-1. Sediment was obtained as described above. The food was added to a test container with a small quantity of water and broken up. An appropriate quantity of sediment slurry was then added and the container filled. The suspension was then sealed and allowed to settle before the small was added. Small were provided with fresh food (0.1 g DW per small) at fortnightly intervals. This quantity of food had previously been established to be in excess of requirements (unpublished data).

Twenty smalls were randomly allocated to six of the treatments, but space limitations allowed only 10 individuals to be used in the seventh (200-1) treatment. Each small was held in an individual 120 ml alabaster container, aerated by means of a whisker bubbler (Hickey & Vickers, 1992), and all were held within the 20°C room under permanent darkness.

Small shell lengths were measured at fortnightly intervals for 12 weeks from 23 December 1996 to 3 March 2000. After being measured, each small was transferred into a fresh container prepared as described above. Proliferation of microbial growths within each container was limited by scraping the sides of the containers with a cloth each of the intervening, alternate weeks. The initial length of these smalls that survived until the end of the experiment in each treatment did not differ significantly (ANOVA, F=0.07, F<0.05), overall mean = 2.03 mm, s=0.053 mm) and growth continued in an approximately linear fashion throughout the experiment. Therefore, growth rates were calculated as the length increase per day and compared between treatments with ANOVA and Tukey’s post hoc test for the difference between means. All statistical analyses were performed in Statview 5.0 and DataDesk 6.0.0.

Results

Assimilation experiment

Assimilation rates for P. antipodorum and Delesseria sp. declined with increasing sediment content in the food mix (Fig. 1). One outlying case is not plotted in Figure 1B. This corresponds to an individual markedly larger than all others (195 mg of DW), which seems likely that residual labelled algae remained adherent to this indi-
virtual after rinsing. This data was included for the purposes of generating the two plots in Figure 1B, but has been excluded from subsequent analyses. Plots of the assimilation rate against the ratio (Food DW/Food DW+Solid-Sediment) show little or no evidence of non-linearity (Fig. 2). Indeed, the slope of this line is almost exactly equal to the mean assimilation rate at zero sediment contamination. There is considerable scatter about this line, particularly at high food contents (low sediment content). We cannot account for this scatter. It could not be reduced by excluding grazers which showed low activity levels during the pre-feeding (i.e. gut-emptying) phase of the experiment, or on the basis of individual size.

**Growth experiment**

Contrary to expectation, within each treatment, growth was approximately linear with respect to time (Fig. 3) rather than being asymptotic. The largest snails reached a final size of approximately 7 mm, and even these showed no sign of slowing growth. Given the linear growth pattern, we determined the growth rate as [(final size - initial size)/experimental duration] for those snails which survived to the end of the experiment. By this measure, sediment contamination had a significant impact upon the long-term growth of snails ($P<0.001$, $F_{1,4}=15.7$, Fig. 4). Surprisingly, mean growth rates (mm d$^{-1}$) were highest at intermediate
Sediment ratio

Growth rate (m·d⁻¹)

Discussion

Our measurements of assimilation were made over a comparatively long period (12-14 h). It is possible that the ¹⁴C content of the periphyton changed over this period (through respiration and photosynthetic fixation of unlabeled CO₂). Algal respiratory rates are usually in the range 0.5-9.0 µmol m⁻² d⁻¹, implying a rather small error over the term of our experiment, however, photosynthetic rates can be up to 2 µmol m⁻² d⁻¹. Fortunately, both high rates would not have occurred in our experiment. The light levels (≈50 µmol m⁻² s⁻¹) at the water surface are unlikely to have been saturating and self-shading would have been very strong at the algal density (60 g DW m⁻²) used in our assimilation experiments. Further more, in all but the zero sediment treatments, most algae would have received almost no light because they would have been further shaded by the sediments with which they were mixed. Thus, we believe that the biomass associated with photosynthetic fixation of unstalled CO₂ during the course of the experiment would also have been minimal.

The short term feeding experiments provide evidence that fine sediment has an adverse influence upon the rates at which P. antipodarum and Delaneola sp. assimilate food. The assimilation rate declines in inverse proportion to the total dry weight of the food-sediment mix. Given that the same quantity of periphyton was added in all treatments, this implies that these two grazers are unable to selectively ingest periphyton when this is contaminated with fine sediments. Rather, they ingest the matrix of periphyton and sediment indiscriminately, and their assimilation rate falls merely because the organic content of this matrix is 'increasingly diluted' by the sediment contamination. Ryder (1989) also found that the ratio of organic matter to sediment in the guts of Delaneola sp. reflected the ratio in the substrates upon which they were feeding. Thus, this appears little doubt that this species cannot preferentially ingest organic material in favour of associated inorganic fines. No\'t it able to compensate for the 'dilution' of the ingested organic material by contaminants by increasing the ingestion rate or the assimilation efficiency.

Ryder (1989) also concluded that the caudalities Phycopetrum sp. is unable to reject inorganic particulates associated with periphyton food, and he also found that their growth rates of the phytoplanktonic Scenedesmus quadricauda and Chlorella oryzae have also been reported to feed non-selectively and suffer impaired growth when sediment contaminates their food (Meth- ting, et al., 1981). Thus, in the case of aquatic insects, the relationship between growth and sediment appears to be negative over the full range of sediment ratios.

In contrast, there is substantial evidence that small brown algae from the presence of low-moderate quantities of sediment in their diet. Simms (1970) found that Laminaria major growth was maximal at intermediate levels of sand contamination, and Jones et al. (2000) came to a similar conclusion for P. antipodarum in Lake Coleridge (South Island, New Zealand). We also found growth to be maximal at intermediate sediment levels in our own long-term growth experiments. It is surprising, therefore, that our short-term assimilation experiments indicate that assimilation is inversely related to the sediment ratio of the food, it may be that over a period exceeding 12 h. P. anti- podarum is able to increase its total ingestion in an
attempt to maintain the rate of ingestion of organic matter despite the presence of sediment contamination. This would at least explain why growth did not decline with rising sediment content from a maximum rate at zero sediment ratio in our long-term growth experiment, though it fails to explain why growth was maximal at intermediate sediment ratios. Such a compensatory response has been demonstrated in Lyhena vulgaris, though it was unaffected within only a few hours rather than over many hours (Anskich, 1993). A more likely explanation for this discrepancy is that P. unioformum is capable of feeding upon the epipelic biofilms which grow upon clay and silt (Lopez & Koehl, 1980). Such biofilms would not have had time to develop in our short-term experiments, and therefore the sediment merely interfered with (delayed) the ingestion of the food periphyton in this study. In our long-term growth experiment the sediment was replaced only at approximately intervals and epipelic biofilms are unlikely to have developed during the intervening 14 days (Lopez & Koehl, 1980). We have shown that P. unioformum is able to harvest such films (even those growing on <63-μm sediment), and if these were more rapidly ingested or assimilated than the presediment primary food (chicken feed, or microbial films growing on the container walls), then ingestion of this epipelic biofilm may explain our finding that most growth was greater at intermediate sediment/primary food ratios than at the zero sediment ratio. It may also be that sediment provided a source of trace minerals or nutrients which were lacking in the primary food supply. None, however, that neither of these mechanisms explain why growth was suppressed at still higher sediment ratios. Anoxic sediments (referred to a change in colour from pale grey to black) were also frequently noted in some of the 1.01 and 5.000 sediment treatments. In these latter treatments the layer of mixed sediment and food was in excess of 2 cm thick, and while the whisker bubbles should have prevented the overlying water from becoming anoxic, it seems that there was insufficient circulation to maintain aerobic conditions in the sediments. Thus, small borrowing for food within the sediment would have been subjected to oxygen stress. This stress would have been compounded by the associated release of sulphides from the matrix of sediment and organic matter. Thus, it may be that the observed increased survival in the high sediment treatments was suppressed as a result of indirect consequences of the high sediment content rather than as a direct result of high sediment content in the diet.

The highest average growth rates in our study were around 11.2 mm d⁻¹. This is higher than rates reported elsewhere in the literature for this species. For example, Duff & Vedder (1995) report a maximum shell growth rate of 2.00 mm d⁻¹ over a 250-d period at 21 °C for snails fed on periphyton growing on sides and DeMello et al. (1995) also measured a rate of 0.03 mm d⁻¹ for snails fed on a mixture of lettuce and lamb's lettuce over 16 weeks at 15 °C. Though confined by the different food sources used (diet temperatures different), comparison of these results might be interpreted as providing further evidence that the presence of some inorganic sediment within the diet promotes the growth of this snail.

Although intermediate levels of sediment were beneficial to the growth of deposit feeding snails, this does not appear to be true of terrestrial grazers in general. Furthermore, high levels of sedimentation (>50%) substantially reduced snail growth rates. Though the mechanisms are unclear, our experiments demonstrated that the sediment contamination levels typical of pasture streams are more favourable to the growth of P. unioformum than are the sediment contamination levels within streams draining altered streams. It is probable that these higher sediment:exposed food ratios in pasture streams contribute to the greater abundance of P. unioformum in pasture streams. Though other differences such as the higher water temperatures (Scarrowbrook unpublished data; Rubenstein et al., 2000) and perhaps also the higher absolute periphyton densities also benefit the snail. Similarly, although the long-term growth data are not available to prove this, our experiments have shown that, in the short-term, Deleatilus sp. is unable to compromise for the 'siltation' of its food with sediments by increasing the efficiency with which it assimilates organic material. Thus, our experiments provide some support for the hypothesis that increased sediment yield in pasture catchments has an adverse impact upon, Drinaeformis sp. and play a role in reducing the abundance of this species in pasture streams relative to altered streams.

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