Effects of the burrowing mayfly, Hexagenia, on nitrogen and sulfur fractions in lake sediment microcosms

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Abstract

Effects of the burrowing mayfly, *Hexagenia,* on nitrogen and sulfur fractions of sediment, and overlying water were determined.Laboratory microcosms were used to reproduce the benthic environment. The activities of *Hexagenia* increased sediment Eh (1.98 ± 0.486 (22) mV · day^{-1}), and decreased pH in sediment (-0.007 ± 0.001 (22) day^{-1}) and overlying water (-0.024 ± 0.004 (10) day^{-1}). In the control, Eh decreased and pH did not change. The presence of *Hexagenia* also markedly increased ammonia in sediment (5.46 ± 0.14 (22) ppm N · day^{-1}) and overlying water (0.792 ± 0.154 (10) ppm N · day^{-1}), while the control did not change. In addition, the sulfate fraction of sediment (0.177 ± 0.006 (17) % dry mass) and water (50.0 ± 4.9 (5) mg · l^{-1}) in microcosms with *Hexagenia* was greater than that of the control (0.151 ± 0.005 (16) % dry mass; 14.7 ± 1.71 (3) mg · l^{-1}) at the termination of the experiment. *Hexagenia* may also stimulate the mineralization of carbon-bonded sulfur. The general role of *Hexagenia* in altering sediment chemistry is discussed.

Introduction

Lake sediments serve as loci for flux and transformation of both nitrogen and sulfur (Keeney 1973; Hornor *et al.* 1980). Nitrogen availability affects lake productivity, while sulfur is important in altering chemical conditions that affect the cycling of other nutrients (Wetzel 1975). Sulfur, in the form of sulfuric acid, is also a major contributor to acid precipitation (Likens *et al.* 1976), and lake acidification (Mitchell *et al.* 1981). Nitrogen chemistry has been studied in freshwater by various investigators (Anderson 1977; Chen *et al.* 1972; Hill 1979), while sulfur has been investigated in much less detail (Stuiver 1967; King & Klug 1980). In addition, little work has been done on the effect of benthic invertebrates on nitrogen and sulfur in freshwater sediments. Recent studies, however, have found that tubificids influence nitrogen transformations in freshwater sediments (Chatarpaul *et al.* 1980) and annelids affect sulfur fractions in marine sediments (Aller & Yingst 1978).

Benthic invertebrates can alter sediment chemistry by a number of mechanisms. Egestion of feces stimulates decomposition and mineralization through translocation, comminution (Kitchell *et al.* 1979) and nutrient enrichment (Hargrave 1976). Bioturbation pumps out nutrient enriched interstitial water and brings in overlying water with a higher concentration of oxygen (Petr 1977). This oxidation alters redox potential (Eh) gradients which are important for determining both the type of chemical transformations and the rate at which they occur (Hornor *et al.* 1980). In addition, bioturbation redistributes sediment particles and disrupts chemical and physical gradients (Krezoski *et al.* 1978; Petr 1977).

The objective of this study was to determine the effect of the nymph of the burrowing mayfly, *Hexagenia,* on sulfur and nitrogen pools, in sediment and overlying water. *Hexagenia* was chosen because of its potential impact on sediment

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chemistry. It is a relatively large insect (up to 5 cm in length), that produces U-shaped burrows which can reach 12 cm below the sediment surface (Edmunds *et al.* 1976). Water currents are pumped through the burrows for gas exchange, and new burrows are continuously dug as the nymphs search the mud for food (Fremling 1967). *Hexagenia* nymphs, therefore, cause marked bioturbation and would be expected to influence sediment chemistry.

Experimental approach

Laboratory microcosms were used to simulate the water–sediment environment. They consisted of 27 glass jars (1.06 l) filled with a homogeneous mixture of five parts sediment to three parts water, obtained in June 1980 from Deer Lake (Essex County, NY) in the Adirondack Mountains. Basic features of the lake are given elsewhere (Mitchell *et al.* 1981). Large debris and macroinvertebrates were removed from the mixture by passing it through a 4 mm sieve. The microcosms were allowed to settle for 2 days, after which the overlying water was gently aerated for the remainder of the experiment. The jars were covered with cheesecloth and deionized water was added each week to compensate for evaporation. The water and sediment were 5 and 8 cm deep, respectively, and had surface areas of 50 cm^2.

Hexagenia nymphs were obtained from Wolf Lake, which is approximately 2 km from Deer Lake, since the former contained higher population densities of mayflies. To each of nine microcosms, two live *Hexagenia* nymphs (~0.1 g wet; *H. limbata* Seville or *H. bilineata* Say) were added. Differentiation between the two species at this stage of development was difficult without damaging the specimens. To another nine microcosms, two dead nymphs killed in boiling water were added to account for possible changes if any *Hexagenia* died during the experiment. An additional set of nine microcosms without *Hexagenia* served as controls. These three sets of microcosms were designated 'Live', 'Dead' and 'Control', respectively. Microcosms were kept in the dark at 20 °C.

An initial sample of homogeneous sediment and overlying water was selected for analysis. After 6, 20 and 54 days three replicates of each microcosm type were also destructively sampled and the sediment was divided into 0–4 and 4–8 cm layers. Sediment samples were stored at 1 °C in sealed plastic bags. All analyses were done on wet samples, with the exception of total sulfur, total nitrogen, sulfate and organic matter, which were determined for dried sediment samples. Water samples were stored at 1 °C in polyethylene bottles.

For all sampling times, the sediment was analyzed for Eh, pH, nitrate, ammonia, Zn-HCl reducible sulfur, HI-reducible sulfur and biomass of the nymphs; the overlying water was analyzed for Eh, pH, nitrate and ammonia. In addition, initial sediment, and samples from day 54 were analyzed for moisture content, organic matter, sulfate, total sulfur and total nitrogen; overlying water samples from day 54 were analyzed for sulfate.

Methods for determining Eh, pH, moisture content, organic matter and nitrate are described in Mitchell *et al.* (1978, 1980). An ion selective electrode was used to determine ammonia concentration (Allen 1974). Total N was determined by the Dumas method with a Coleman® nitrogen analyzer (Sternglanz & Kollig 1962). Sulfate was determined using the barium chloranilate procedure (Johnson & Henderson 1979), and total sulfur was found by the method of Steinbergs *et al.* (1972). Zn-HCl reducible sulfur (inorganic non-sulfate sulfur) and HI-reducible sulfur (inorganic sulfur and ester sulfate) procedures were performed by methods described previously (Waugh & Mitchell 1981). All sediment analyses are reported on a dry mass basis.

Results and discussion

At the termination of the experiment, no mortality had occurred and a linear nymphal growth response was observed ($r = 0.99$; Fig. 1). Numerous burrows were visible through the sides of the Live microcosms throughout the study. By the last sampling date (day 54) wing pad development indicated that the nymphs were nearing emergence.

Statistical analysis of the data was performed by analysis of variance (ANOVA), regression and interval estimates of the mean. The significance of microcosm type (Live, Dead, Control) sediment depth (0–4, 4–8 cm) and time were determined by ANOVA. Sediment depth for all analyses except sulfate was not significant ($p > 0.05$) and with this

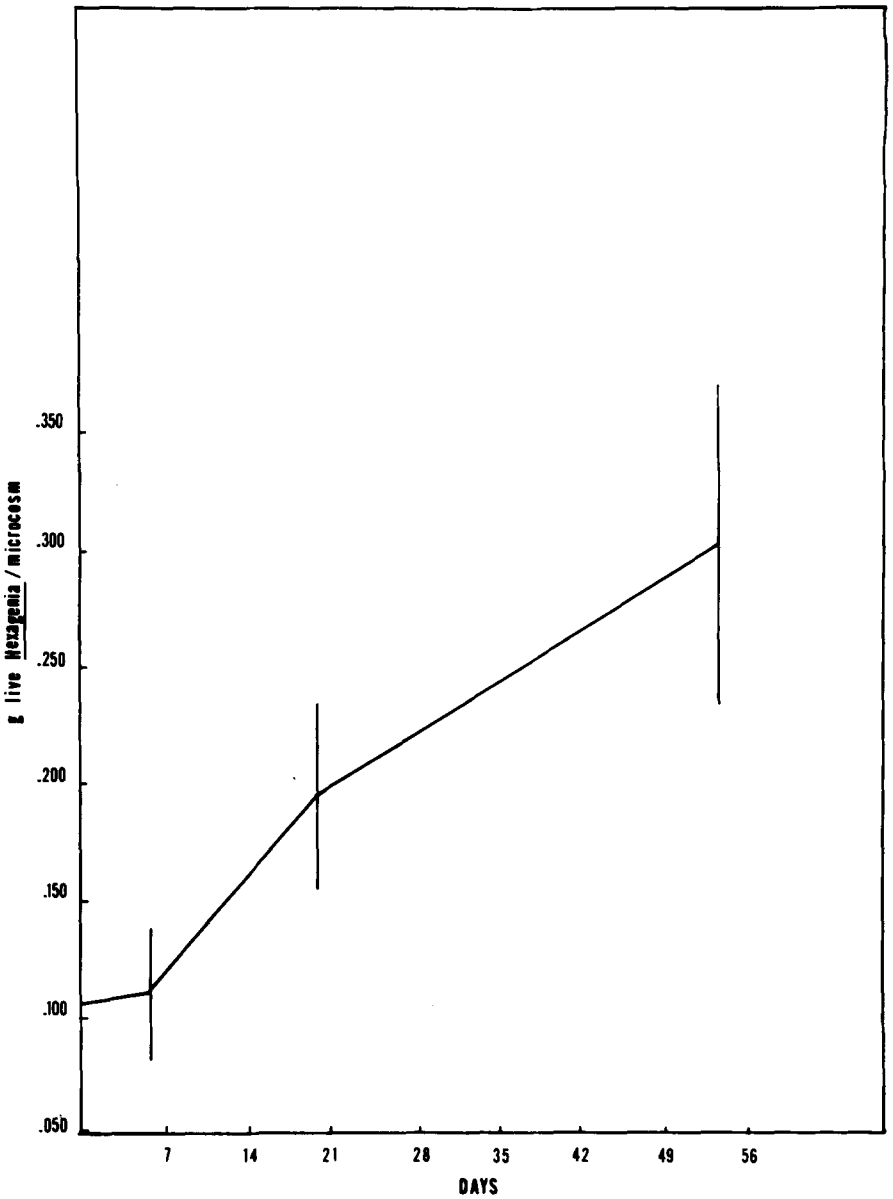

Fig. 1. Mass of *Hexagenia* nymphs in Live microcosms (means with standard errors; n = 3).

exception, the data for the depths were combined.

No differences ($p > 0.05$) were found between microcosm types for organic matter, total sulfur and total nitrogen. This similarity indicates the homogeneity of the sediment used for the experiment. Values for the characteristics which did not vary ($p > 0.05$) among microcosms and did not change with time, are listed in Table 1.

Eh and pH

Sediment Eh increased markedly with time ($p < 0.01$; Fig. 2) in the Live (1.98 ± 0.486 (22) mV · day^{-1}; regression coefficient ± S.E. (D.F.)), showed no change ($p > 0.05$) in the Dead, and decreased in the Control ($p < 0.01$; -1.74 ± 0.545 (22) mV · day^{-1}). Overlying water Eh increased with time ($p <$

Table 1. Characteristics which varied with neither ($p > 0.05$) microcosms nor time.

Sediment characteristic*	Mean	S.E.	(N)
% dry matter	12.1	± 0.315	(19)
% organic matter	27.9	± 0.218	(19)
% total nitrogen	1.12	± 0.0113	(19)
% total sulfur	0.677	± 0.059	(19)
% HI-reducible sulfur	0.20	± 0.06	(19)
Water characteristic			
ppm nitrate	0.995	± 0.302	(28)

* Dry mass

0.01; 1.95 ± 0.410 (34) mV · day^{-1}), undoubtedly due to the aeration, but there were no differences between types ($p > 0.05$).

In the Live, pH in both sediment (Fig. 3; -0.007 ± 0.001 (22) day^{-1}) and overlying water (Fig. 4; -0.024 ± 0.004 (10) day^{-1}) decreased ($p < 0.01$). In the Dead, the sediment pH increased ($p < 0.05$; 0.004 ± 0.001 (22) day^{-1}) while the overlying water pH decreased ($p < 0.01$; -0.018 ± 0.005 (10) day^{-1}). The pH in the Control did not change ($p > 0.05$) in either sediment or water.

Similar responses in Eh and pH have been

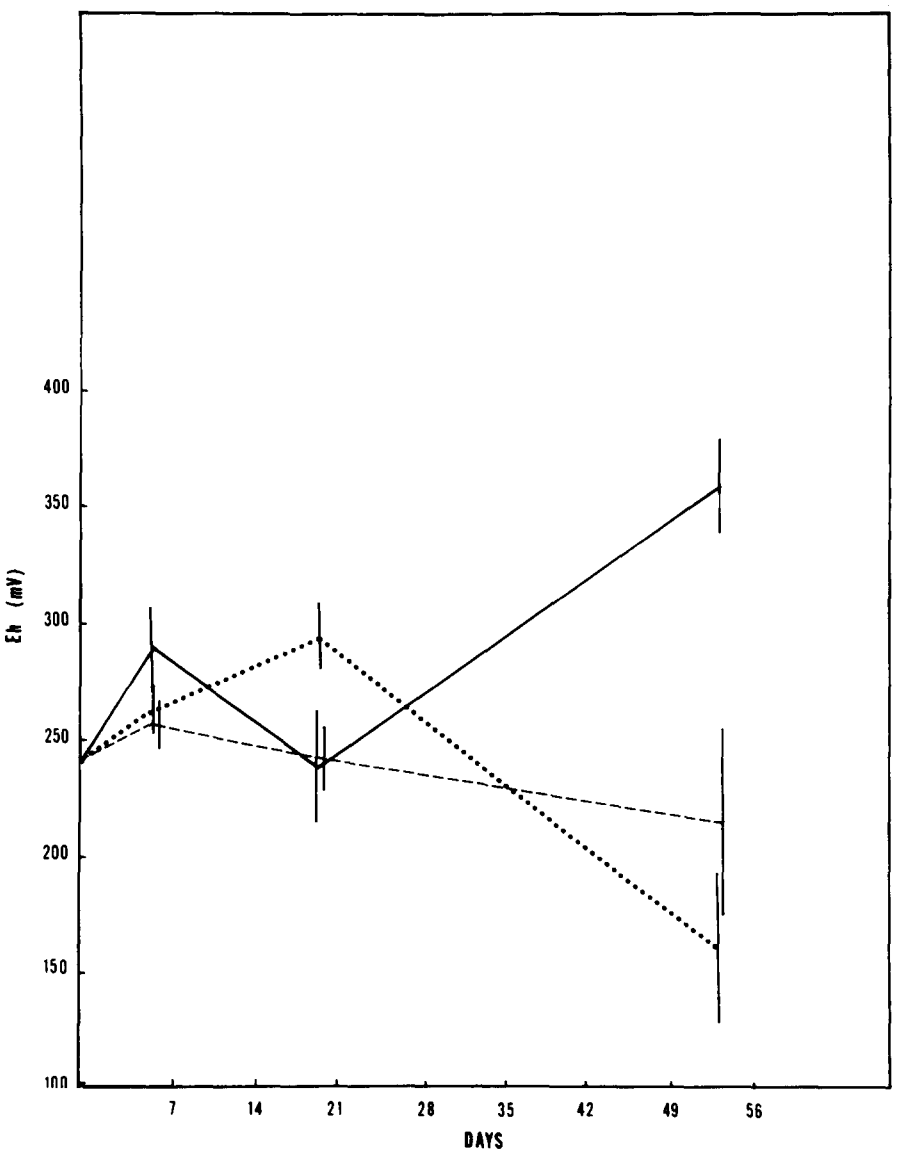

Fig. 2. Redox potential (Eh) in sediment of Live (solid lines), Dead (dashed lines) and Control (dotted lines) microcosms. Means with standard errors (vertical lines); n = 3.

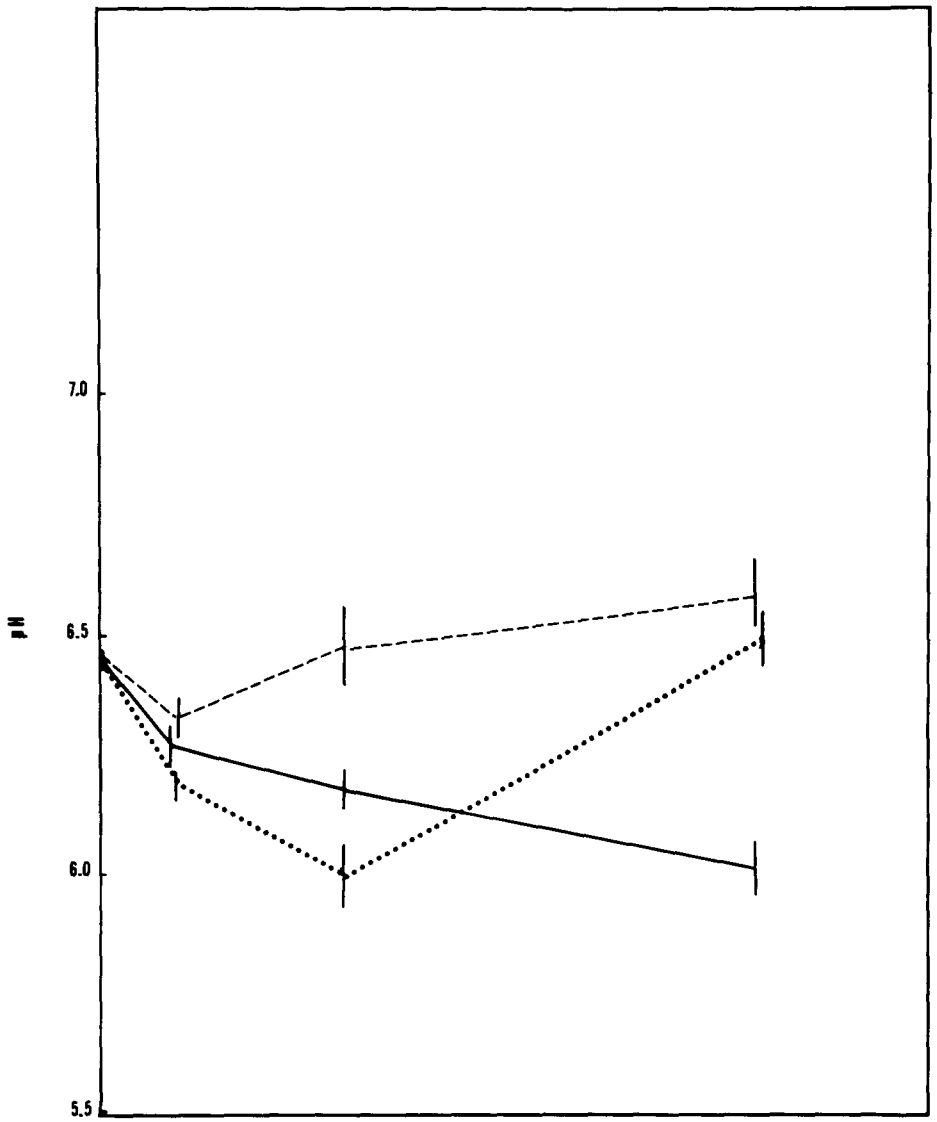

Fig. 3. Sediment pH of Live (solid lines), Dead (dashed lines) and Control (dotted lines) microcosms. Means with standard errors; n = 6.

attributed to chironomids and tubificids in sediment (Graneli 1979; Davis 1974). The *Hexagenia* nymphs, by actively pumping currents through their burrows, increased oxygen penetration which altered the sediment redox potential. Higher dissolved oxygen concentrations in the sediment could stimulate metabolic actively, and increase the production of organic acids and carbon dioxide. This could cause the observed decrease in sediment pH. The decrease in pH in the overlying water in the Dead was probably related to the decomposition of the dead *Hexagenia* on the sediment surface.

Nitrogen components

In the Live, ammonia increased markedly ($p < 0.01$) in both the sediment (Fig. 5; 5.46 ± 0.514 (22) ppm N · day^{-1}) and the overlying water (Fig. 6; 0.792 ± 0.154 (10) ppm N · day^{-1}). Ammonia in the Dead and Control did not show a change ($p > 0.05$)

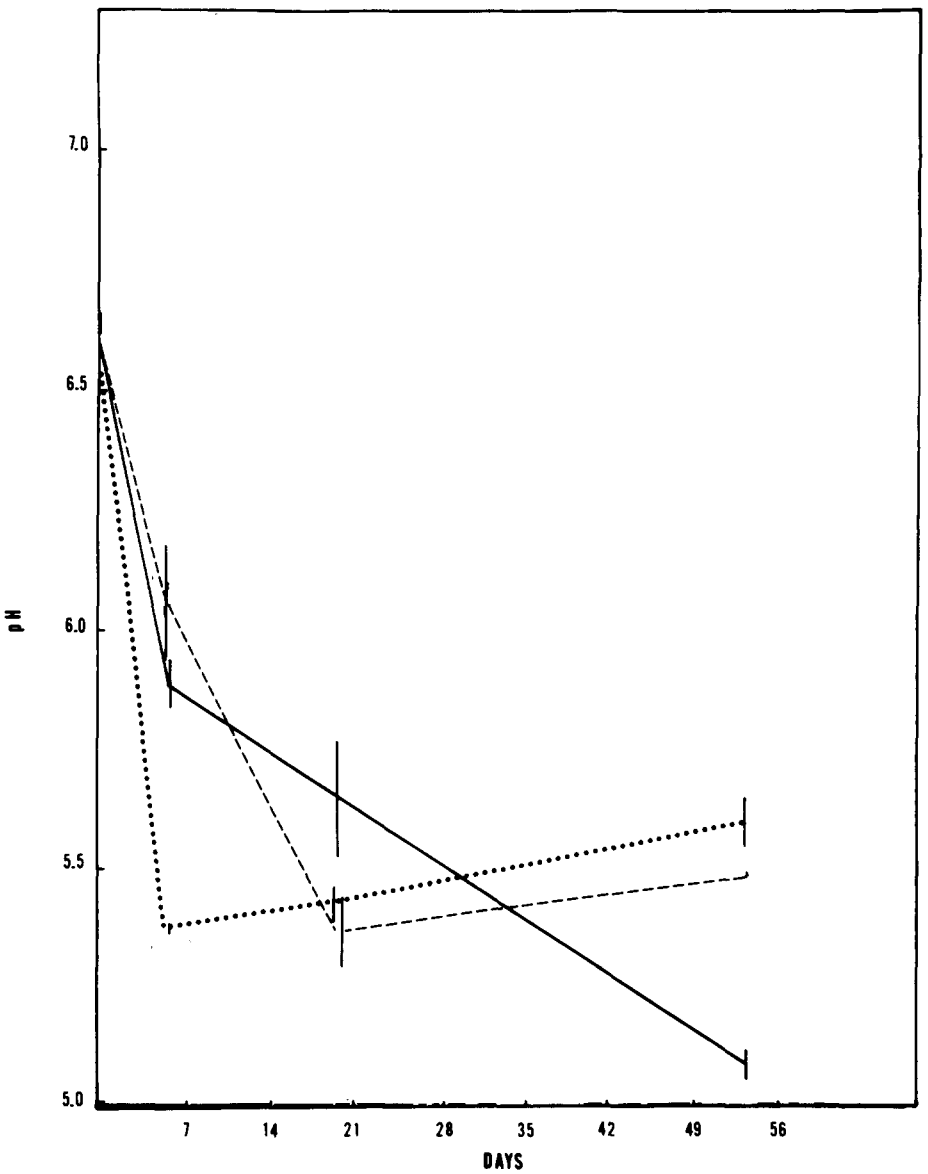

Fig. 4. Overlying water pH of Live (solid lines), Dead (dashed lines) and Control (dotted lines) microcosms. Means with standard errors; n = 3.

in the sediment or water. Nitrate in the sediment increased ($p < 0.05$) in the Live (0.202 ± 0.081 (22) ppm N · day^{-1}) and the Control (0.107 ± 0.05 (22) ppm N · day^{-1}), while no change ($p > 0.05$) was found in the Dead sediment or in the overlying water of the three types.

Previous studies have shown the effect of invertebrates on sediment nitrogen cycling to be complex. Chatarpaul *et al.* (1980) found that tubificids accelerate both denitrification and nitrification in stream sediments. Edwards (1958) found that chironomids increased ammonia and decreased oxidized nitrogen in water overlying settled activated sludge. Anderson (1977) also observed an increase of denitrification in overlying water after addition of chironomids. In the Live microcosms, nitrification of ammonia may have contributed to the increase in sediment nitrate (Chatarpaul *et al.*

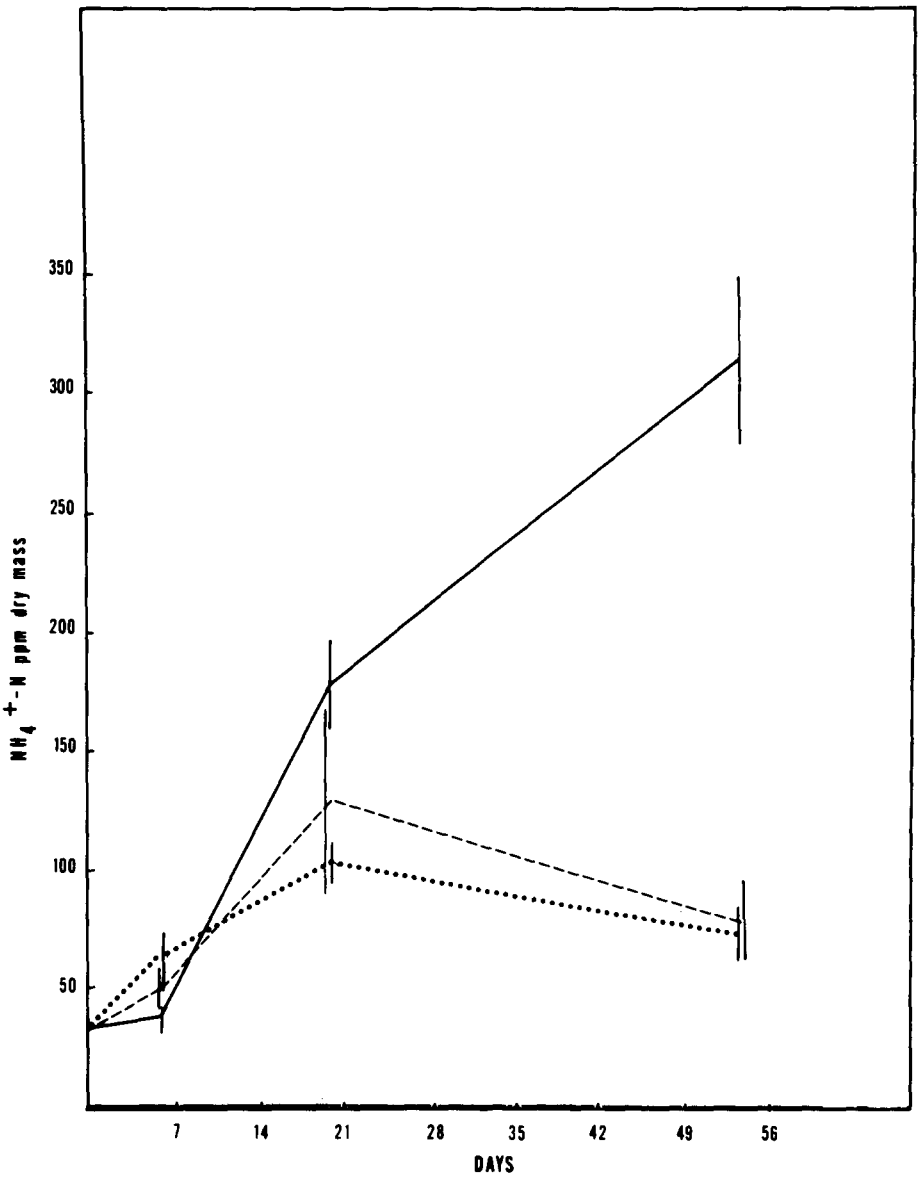

Fig. 5. Sediment ammonia in of Live (solid lines), Dead (dashed lines) and Control (dotted lines) microcosms. Means with standard errors; n = 6.

1979). Circulation of oxygenated water through the burrows could stimulate this process. The reason for the increase in nitrate in the Control is unknown.

The rapid increase of ammonia in the Live was undoubtedly related to excretion by the nymphs. The rate of ammonia production in the Live after the value for the Control was subtracted equaled 11.83 mg N · g wet larvae^{-1} · day^{-1}. This, however, is an order of magnitude greater than the rate of ammonia produced by excretion for *Chironomus riparius* 0.37 mg N · g wet larvae^{-1} · day^{-1} (Edwards 1958). The relationship between *Hexagenia* and sediment ammonia warrants further study.

Sulfur components

Zn-HCl (reduced inorganic sulfur) decreased in all types (Fig. 7; $p < 0.05$), with the Live showing

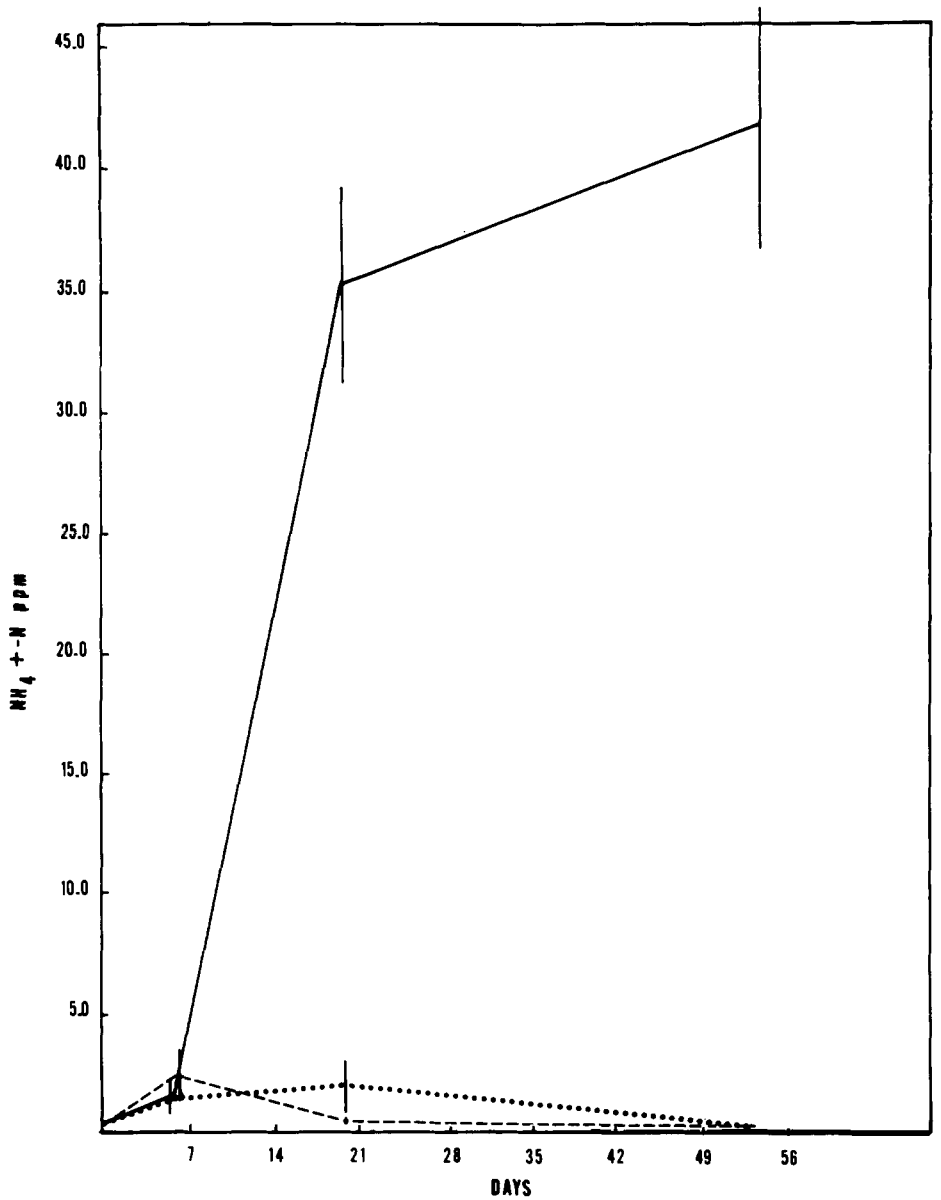

Fig. 6. Ammonia concentration in overlying water of Live (solid lines), Dead (dashed lines) and Control (dotted lines) microcosms. Means with standard errors; n = 3.

the most rapid rate of decrease. The rates were -7.1 ± 1.5 (22) ppm S · day^{-1}, -5.5 ± 1.7 (22) ppm S · day^{-1}, and -4.0 ± 1.8 (22) ppm S · day^{-1} for Live, Dead and Control, respectively. A general increase in HI-reducible sulfur for all microcosms ($p < 0.01$; inorganic sulfur and ester sulfate) was also found, with the Live showing a higher rate (7.2 ± 6.9 (22) ppm S · day^{-1} than the Dead (0.7 ± 3.3 (22) ppm S · day^{-1}) and the Control (4.7 ± 4.4 (22) ppm S · day^{-1} although the differences among microcosm types were not significant ($p > 0.05$). Sulfate increased with time ($p < 0.05$) in all types and depths of sediment except the top depth of the Dead (Table 2). On day 54 sediment sulfate in the Live was greater ($p < 0.05$) than in the Control or the top depth of the Dead. Sulfate in the overlying water was also greater ($p < 0.01$) in the Live than the Dead or Control.

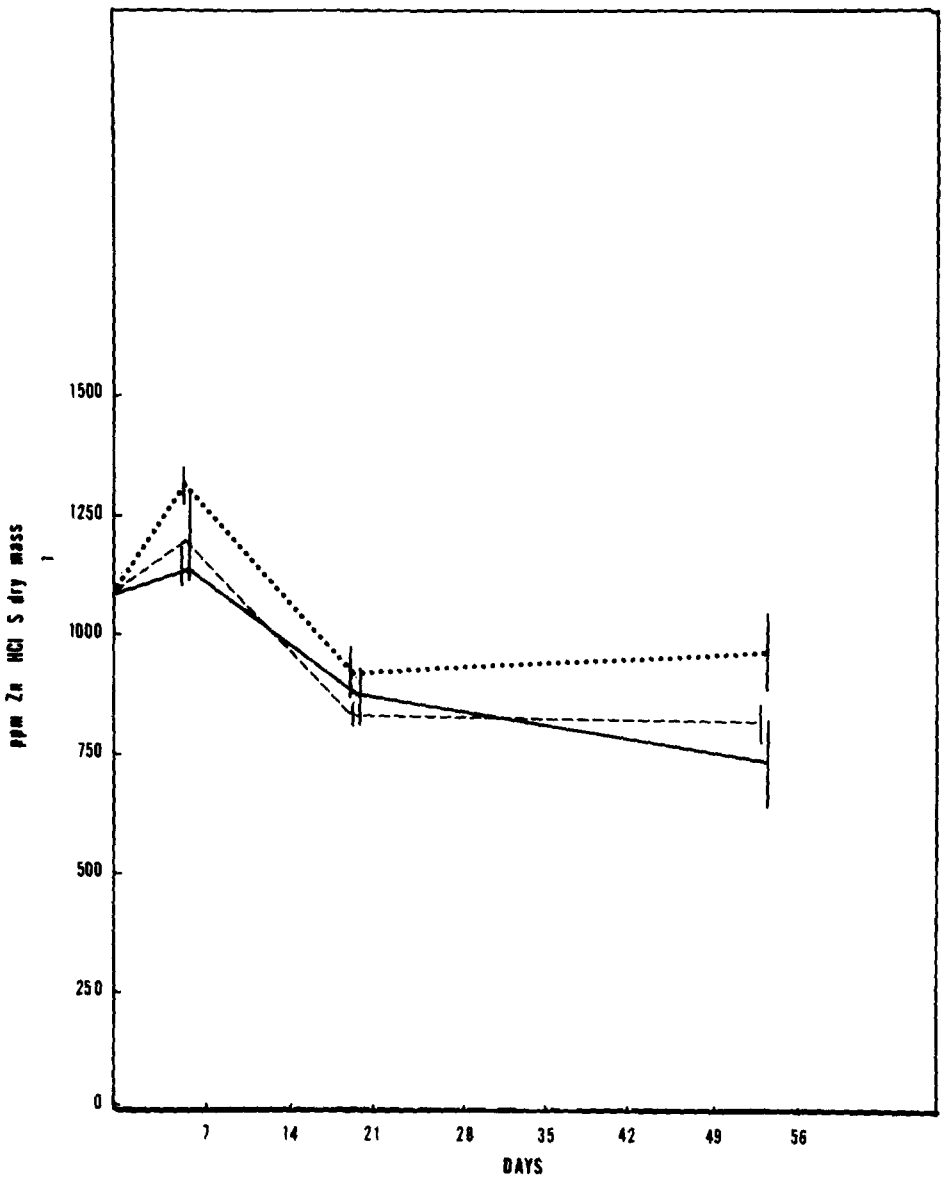

Fig. 7. Inorganic reduced sulfur compounds (Zn-HCl S) of Live (solid lines), Dead (dashed lines) and Control (dotted lines) microcosms. Means with standard errors; $n = 6$.

The results of the sulfur analysis indicate that *Hexagenia* may be affecting sulfur dynamics in two ways. Their activities cause a general increase in the oxidation state of sediment sulfur species, shown by the decrease in Zn-HCl sulfur and concurrent increase in sulfate. They may also be be stimulating decomposition and mineralization rates. Total sulfur remained constant in the experiment. Since carbon bonded sulfur is the difference between total sulfur and HI-reducible sulfur, the higher rate of increase of HI-reducible sulfur in the Live suggests that *Hexagenia* may increase decomposition rates.

Summary and conclusions

The burrowing activities of *Hexagenia* nymphs markedly affect sediment chemistry. By circulating

Table 2. Microcosm sulfate fractions in % dry mass for sediment; ppm for overlying water. A significant difference was found between depths ($p < 0.05$) in the Dead, so they were not combined.

Sample type	Time (day)	Mean	S.E.	(N)
Initial sediment	0	0.100	± 0.008	(3)
Sediment of Live*	54	0.177	± 0.006	(18)
Sediment of Dead (top depth)*+	54	0.119	± 0.007	(9)
Sediment of Dead (bottom depth)*	54	0.162	± 0.007	(9)
Sediment of Control*+	54	0.151	± 0.005	(17)
Water of Live	54	50.0	± 4.9	(6)
Water of Dead+	54	5.7	± 2.9	(7)
Water of Control+	54	14.7	± 1.71	(4)

* Sulfate that changed with time.
\+ Sulfate that was significantly different ($p < 0.05$) from the Live, by interval estimates.

water through their burrows the nymphs alter the sediment redox potential. The effect of this is shown by an increase in the pools of oxidized sulfur and nitrogen. The more aerobic environment provided by the nymphs may also stimulate microbial activity and decomposition rates. In addition, *Hexagenia* nymphs also increased ammonia and decreased pH in both sediment and water. These effects are probably related to the excretory products of the animals.

It is important that in each case in which the presence of *Hexagenia* altered the sediment chemistry, a similar change was also found in the overlying water, with the exception of nitrate. This points out an additional effect of *Hexagenia* bioturbation. Materials were exchanged between the sediment and overlying water by mass flow in addition to diffusion. In this manner exchange rates were greatly increased and the overlying water of the microcosms reflected the changes occurring in the sediment. This mechanism increases the recycling of nutrients and thus stimulates production.

This is the first study to consider the effects of benthic invertebrates on sulfur in freshwater sediment. Further work is needed to delineate the chemical pathways involved in both sulfur and nitrogen transformations in sediments and the role of benthic invertebrates in these processes. This information will provide a better understanding of nutrient cycling in limnetic systems.

Acknowledgements

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