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Seasonal relationships between planktonic microorganisms and dissolved organic material in an alpine stream

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Abstract. The relationships between the abundance and activity of planktonic, heterotrophic microorganisms and the quantity and characteristics of dissolved organic carbon (DOC) in a Rocky Mountain stream were evaluated. Peak values of glucose uptake, 2.1 nmol L^{-1} hr^{-1}, and glucose concentration, 333 nM, occurred during spring snowmelt when the water temperature was 4.0 °C and the DOC concentration was greatest. The turnover time of the *in situ* glucose pool ranged seasonally from 40—1110 hours, with a mean of 272 hr. Seasonal uptake of ^{3}H-glucose, particulate ATP concentrations, and direct counts of microbial biomass were independent of temperature, but were positively correlated with DOC concentrations and negatively correlated with stream discharge. Heterotrophic activity in melted snow was generally low, but patchy. In the summer, planktonic heterotrophic activity and microbial biomass exhibited small-scale diel cycles which did not appear to be related to fluctuations in discharge or DOC, but could be related to the activity of benthic invertebrates. Leaf-packs placed under the snow progressively lost weight and leachable organic material during the winter, indicating that the annual litterfall in the watershed may be one source of the spring flush of DOC. These results indicate that the availability of labile DOC to the stream ecosystem is the primary control on seasonal variation in heterotrophic activity of planktonic microbial populations.

Introduction

In many stream ecosystems, the flux of DOC is greater than the flux of other forms of organic material, such as coarse or fine particulate organic material. The assumption that most of this DOC is not readily utilized by heterotrophic microorganisms has been challenged by several recent studies (Meyer 1990; Vervier & Naiman 1992). Because of the magnitude of the DOC flux, microbial DOC uptake could be significant in carbon

cycling for stream ecosystems (Meyer 1990). Three factors that make the study of DOC cycling in stream ecosystems difficult are (1) the potential differences between microbial populations in the epilithon and in the flowing streamwater, (2) the complexities of the hydrologic flowpaths in the watershed and the hyporheic zone and (3) the heterogeneous nature of organic compounds comprising the DOC.

The objective of the study presented here was to evaluate the relationships between the abundance and activity of heterotrophic microorganisms and the quantity, characteristics and sources of DOC in Deer Creek, a Rocky Mountain stream. We studied microbial activity in the streamwater rather than in the epilithon because a previous study of the Red Deer River in Alberta (Baker et al. 1982; Baker 1986) had shown that microbial activity in the epilithon was fairly constant throughout the year but planktonic microbial activity followed a seasonal pattern closely tied to the large spring influx of DOC. Deer Creek is similar to other mountain streams and to the Red Deer River in that maximum DOC concentrations occur during early snowmelt, which can be explained by flushing into streams of DOC-enriched soil interstitial water, with riparian zone soils of particular importance (Denning et al. 1991; Meyer 1990; Easthouse et al. 1992; Hornberger et al. submitted). However, in Deer Creek the streamwater temperature is very low (0.25—4.5 °C) during snowmelt which could limit heterotrophic activity.

In Deer Creek, as in other streams, dissolved humic substances account for a large portion of the DOC, especially during spring snowmelt (McKnight et al. 1992; Aiken et al. 1992). Identifiable organic substrates, such as glucose, which may be very labile compared to humic substances, are typically present in lower concentrations (Thurman 1985).

Several approaches were used to characterize seasonal changes in planktonic microbial populations in Deer Creek, including particulate ATP concentrations, direct counts, and the rate of glucose uptake. Discharge, stream temperature and concentrations of DOC and glucose were also followed. Short term studies of diel variations in microbial activity, microbial activity in snowpack and leaching of litterfall under the snowpack were conducted to aid in interpretation of results.

Site description

The study was conducted in Deer Creek, a small Rocky Mountain headwater stream located near the town of Montezuma in Summit County, Colorado (Fig. 1). The sampling site was in a smooth flowing reach about 20 m above the confluence of Deer Creek with the Snake River (Fig. 1).

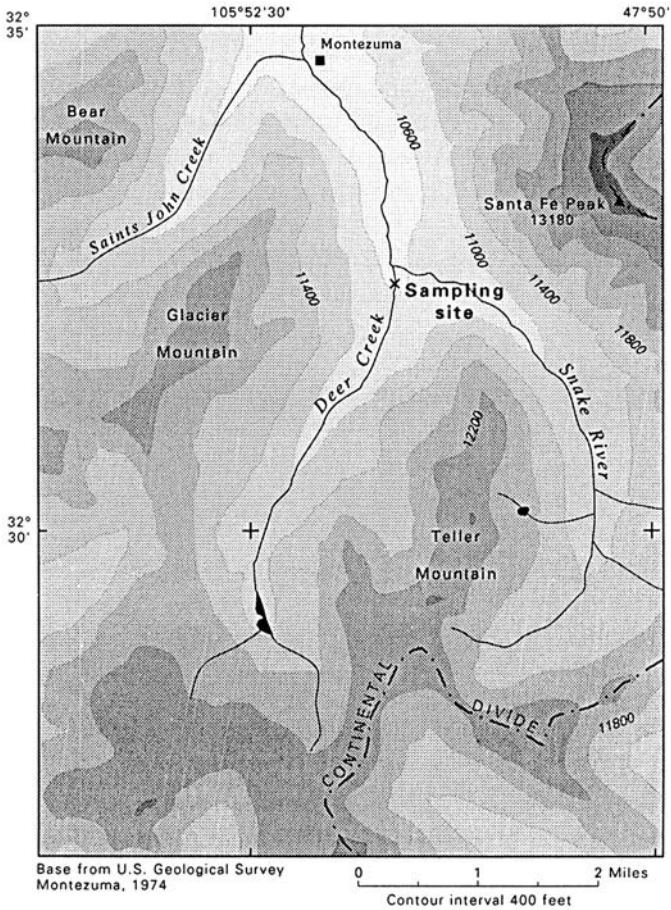

Fig. 1. Topographic map of Deer Creek watershed, showing sampling site upstream from the confluence with the Snake River.

Water samples were collected approximately 0.5 m from the edge of the bank where water depth ranged from 15 cm to 50 cm during spring runoff. The stream is bounded by a low willow marsh at the site and is not shaded by trees for several hundred meters upstream. Beaver are active in the area and have constructed dams on the stream and in a nearby marsh.

The Deer Creek watershed was described in detail by Theobald et al. (1963). The basin drains an area of about 10 km^2 and has an outlet at the confluence with the Snake River at an elevation of 3,260 m. The streambed is composed of mica-rich quartz sands and well-rounded rocks that have a hard black iron and manganese coating. Sandbar willows (salix) and sedges are abundant in the riparian zone. The upland forests

are dominated by Colorado blue spruce (*Picea pungens*), Englemann spruce (*Picea englemannii*), Subalpine fir (*Abies lasiocarpa*), and Lodgepole pine (*Pinus contorta*). In the central third of the stream the riparian zone consists of extensive marshes and pools. During the summer the periphyton is composed primarily of diatoms (e.g. *Hannea arcus*) and the most abundant benthic invertebrates are mayflies (McKnight & Feder 1984).

The hydrologic cycle in the Rocky Mountains is dominated by the melting of snowpack in the spring (May—June), which typically causes 10-fold increases in discharge. After snowmelt, minor increases in streamflow are caused by afternoon thunderstorms. During snowmelt many solutes decrease in concentration because of dilution (Lewis & Grant 1979), but DOC concentrations increase 2—4 fold (McKnight & Bencala 1990; Baron et al. 1991). The maximum DOC concentration typically precedes maximum discharge by several weeks (McKnight et al. 1992).

Sampling and analytical methods

Deer Creek was sampled weekly from May to August in 1984 and from April to August in 1985 and was sampled monthly during the intervening winter. Extreme care was exercised to prevent disruption of the streambed. On September 9—10 and November 7—9, 1984, diel variations were monitored at about 4 h intervals. The September study was begun following an afternoon thunderstorm. During the November study, a snowstorm began before noon on November 8, 1984. In 1985 samples were collected in late morning between 10:30 A.M. (MDT) and noon. Stream stage height was recorded and discharge calculated using a rating curve developed from streamflow measurements.

Samples for inorganic analysis were filtered through 0.4 μm Nuclepore* filters using an Antlia filtration unit and collected in 50 mL acid-washed, distilled water-rinsed, plastic bottles that had been rinsed 3 times with streamwater. Anions were analyzed using a Dionex ion chromatograph. Cation samples were acidified with 0.5 mL of Ultrex nitric acid and analyzed using a Jarrel-Ash 975 inductively coupled plasma spectrometer. DOC samples were filtered through 0.4 μm Selas silver membrane filters using a Gelman stainless steel pressure filtration unit and collected in precombusted glass bottles. DOC analysis was done in duplicate by Huffman Laboratories, Wheatridge, Colo. using a high temperature com-

* The use of trade or firm names is for identification purposes only and does not imply endorsement by the US Geological Survey.

bustion (950 °C) technique (Coulmetrics, System 130 with a combustion tube containing tungsten trioxide, silver, and barium chromate). Silver membrane filters were analyzed for suspended organic carbon (SOC) by the US Geological Survey National Water Quality Laboratory (USGS-NWQL) using an ampule method with an Oceanographic International carbon analyzer. Samples for glucose analysis was filtered through 47 mm diameter, 0.4 μm Nuclepore filters, collected in 3.8 L plastic cubitainers, and frozen. Glucose was analysed by Huffman Laboratories following the method of Cavari & Phelps (1977) without the preconcentration step and using a Turner model TD-20e Luminometer. Standard curves were determined daily and standards were analysed every eighth sample. The day-to-day variation was +/− 20% for duplicate samples in the 50—300 nmol L^{-1} range.

During 1984, measurements were made of particulate adenosine triphosphate (ATP) concentration and ^{3}H-glucose uptake. ATP was extracted from bacteria following a modification of the method of Bulleid (1978). A 1 L water sample was filtered through a 0.45 μm Gelman GN6 filter, and the filter was boiled for 6 min in 8 mL of 0.04 M Na_2HPO_4 buffer adjusted to pH 7.7 with 0.02 M citric acid. For the first min of boiling, the beaker was placed directly on a hot plate; for the last 5 min the beaker was in a boiling water bath. The cooled extract was brought to 10 mL volume with distilled water, while rinsing the filter, and then diluted 1:1 with 0.1 M tris buffer at pH 7.7. The extract was either assayed immediately or stored in a plastic vial at −4 °C. ATP was quantified with a luciferin/luciferase preparation (FLE 50, Sigma Chemical Co.). Fifty mg of lyophilized FLE 50 was rehydrated for 1 h at 4 °C in 17.5 mL of distilled water, 7.5 mL of 0.1 M $KAsO_4$ (pH 7.45) and 5 mL of 0.04 M $MgSO_4$. A plastic scintillation vial containing 0.5 mL of sample and 0.5 mL of rehydrated luciferin/luciferase (added immediately prior to analysis) was placed in the chamber of a Diagnostic Sciences model 3000 integrating ATP photometer and light emission was measured. Internal ATP standards added during boiling were used to assess ATP recovery and duplicates with internal standards were run for each sample.

Uptake of tritiated glucose by bacteria was determined in sterile syringes in a water bath maintained at *in situ* stream temperature in the dark. Four 10-mL aliquots of each sample were placed in plastic 10-mL syringes, sealed with rubber-stoppered injection hubs. In 1984, another set of 4 10-mL syringes were enriched to a glucose concentration of 5 μM. All syringes were injected with ^{3}H-glucose (14.5 Ci/mmol, 0.187 uCi total), inverted several times and incubated in the water bath. After approximately 4 h of incubation, the contents of each syringe were filtered through a 0.45 μm, 25 mm diameter Gelman GN6 filter. The filters were

rinsed twice with 10 mL ice cold, filter-sterilized streamwater. The filters were dissolved in 1 mL ethyl acetate; 10 mL of Beckman EM scintillation fluor was added and the sample was assayed for ^3H activity by liquid scintillation counting with internal standard addition (^3H-toluene) for quench correction. Activity in formaldehyde-killed controls indicated negligible ^3H-glucose adsorption. Comparison of 0.45 μm filters to 0.2 μm filters showed that 80% of the glucose-utilizing bacteria was retained by the 0.45 μm filter. Time course studies (0—4 hours) were done as well in 1984. Glucose turnover time was calculated by dividing the concentration of added ^3H-glucose by the ^3H-glucose uptake rate (Hobbie 1967).

The diel study on November 7—9, 1984 included measurements of chlorophyll content and benthic invertebrate drift. Samples (2 L) were filtered through 47 mm GFC glass fiber filters and analyzed for chlorophyll by the USGS-NWQL. Drifting benthic invertebrates were collected at mid-stream with a drift net for approximately 30 mins. During the snowstorm, the drift net site was moved upstream, but comparable results were obtained for samples collected at the two sites on the final sampling point. Benthic invertebrates were enumerated by Chadwick and Associates, Littleton, CO.

In 1985, epifluorescent direct counts of bacteria (Smith et al. 1991) were determined and algae were enumerated by Chadwick and Associates. On May 5, 1985 and February 20, 1986 snow was collected at a site 30 m from the stream at depths 15 to 30 cm below the snowpack surface; the snowpack was about 1 m deep. Sterile implements were used to dispense the snow into buckets lined with sterile plastic bags. After melting for 36 h at room temperature, the snow samples were processed in the same manner as streamwater samples and analyzed for DOC and ^3H-glucose uptake.

Over the winter of 1984—1985, leaching of DOC from leaves under the snowpack was studied. Willow leaves were collected in October, stitched into nylon mesh packs, freeze-dried and then weighed. On October 26, 1984, 40 preweighed leaf packs were anchored at sites in the riparian zone (about 20 m from the stream). Several leafpacks at a time were retrieved throughout the winter and spring of 1985. The leafpacks were packed in dry ice in the field and freeze-dried, reweighed and stored in sterile, precombusted glass jars.

Leachable organic material was quantified by adding to each leafpack container a measured amount of distilled water (700—900 mL), leaching for 6—10 h at 4 °C, and then decanting and filtering the leachate through a precombusted GFC glass fiber filter. The samples were filtered over a 4 h period, and the 3—6 samples retrieved on each date were filtered at different times during that period. DOC concentrations in the leachates

were analyzed by the USGS-NWQL using a Dohrmann carbon analyzer. The duration of leaching did not influence the leachate DOC concentrations. Glucose concentration was measured with dilutions of 1:500 or 1:625. The leachates from the same retrieval date were combined, acidified and 700 to 1000 mL was passed through a XAD-8 column (Thurman & Malcolm 1981). The concentration of dissolved hydrophobic neutrals and acids was determined from the difference between influent and effluent DOC concentration. The XAD-8 column was eluted with 0.1 N NaOH; the eluate was H^+-saturated using a cation exchange resin and freeze-dried. The concentration of dissolved fulvic acid was determined from the weight of the freeze-dried sample by assuming a carbon content of 50%.

The 1984 and 1985 data sets were analyzed by stepwise multiple regression using the Minitab software (Minitab 1988). In this analysis, a greater absolute value of the t-ratio corresponds to a greater probability that the correlation did not occur by chance. For t-ratios with an absolute value greater than 2, the corresponding probability is greater than 95%. The dependent microbial variables were ATP concentration, tritiated glucose uptake rate constant, glucose uptake rate, and direct bacterial counts. Each dependent variable was regressed against a set of independent variables consisting of temperature, discharge, DOC concentration, and, for 1985, glucose concentration. The regression was repeated excluding temperature because in the initial analysis the t-ratio for temperature was much less than 2.

Results and discussion

Seasonal variations in microbial populations and activity

Glucose is commonly chosen as a substrate to measure microbial heterotrophic activity in an aquatic system because it can be utilized by a wide range of microorganisms (Wright & Hobbie 1966). Although *in situ* glucose concentrations constitute only a small fraction of the total DOC, glucose is often the predominant free carbohydrate present in water (Thurman 1985) and therefore represents the more labile components of the DOC pool. Glucose is also an intermediate in the decomposition of cellulosic material, a major component of plant litter in the watershed. In 1984 rates of tritiated glucose uptake were determined during 4-h incubations (Fig. 2); 4 h is somewhat less than the travel time through Deer Creek. All of the incubations resulted in linear uptake with time; the standard error of the calculated slopes ranged from 4—28% for the *in situ* glucose concentration incubations. Because uptake was linear, only a 4-h

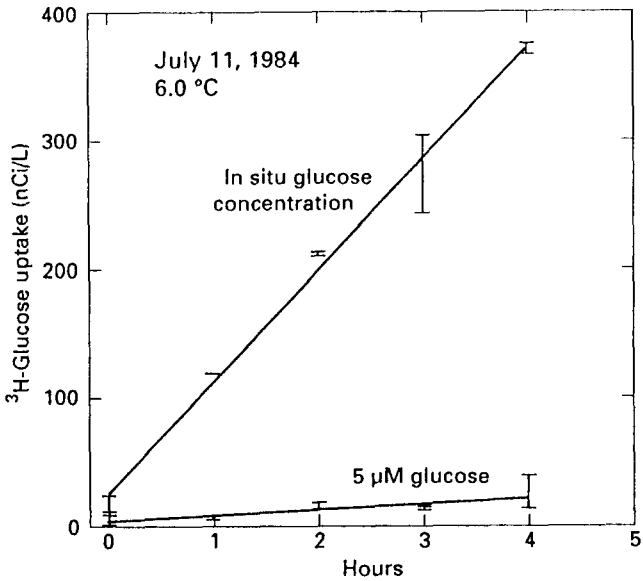

Fig. 2. Time course of ^{3}H-glucose uptake in Deer Creek water samples when incubated at the *in situ* temperature. Points are means of replicate samples; error bars are ± one standard deviation.

incubation time was used in 1985. The *in situ* glucose uptake rates for Deer Creek appear to be low (Table 1) and the turnover time of the *in situ* glucose pool ranged from 40—1110 h (mean 272 h), these turnover times are much greater than the travel time through Deer Creek.

Few other studies have simultaneously measured glucose concentrations and uptake rates. The turnover times in Deer Creek are within the range reported by Goulder (1984) for a calcareous headwater stream (71—333 h), higher than turnover times in the Okefenokee Swamp (seasonal mean of 110 h in the water column (Murray & Hodson 1984)), and considerably higher than glucose turnover times in several eutrophic waters (3—46 h (as reported in Gocke et al. 1981)). A comparison of turnover times must be made with caution, because this parameter may change with *in situ* glucose concentration. For an effective comparison, in 1984 glucose uptake was assessed under enriched conditions (5 μm glucose), which masks changes in the *in situ* glucose pool, and the rate only varied 7-fold (0.2—2.9 nmol L^{-1} hr^{-1}, Table 1). On three of the four occasions for which the *in situ* glucose concentration was determined, the glucose uptake rate at 5 μm glucose was not different from the *in situ* rate (Table 1).

In 1985 *in situ* glucose concentrations were determined for all but

three of the sampling dates. Values ranged from below detection limit (11 nM) to 333 nM, with a mean for the year of 97 nM. The highest glucose concentration represented 1.8% of the total DOC. Other studies have found glucose concentrations to be 10—1000 nM (Vaccaro et al. 1968) and 2—400 nM (Mopper et al. 1980) in seawater, 400—800 nM in eutrophic, brackish and fresh waters (Gocke et al. 1981), and 110 nM in Lake Kinneret (Cavari & Phelps 1977). Unlike the glucose concentration, the *in situ* glucose uptake rate in Deer Creek was much more constant seasonally, varying from 0.2—2.0 nmol L^{-1} hr^{-1}. There was a linear correlation ($r^2 = 0.8$) between the uptake rate and the *in situ* glucose concentration. When normalized for bacterial abundance, the specific glucose uptake rate was 0.24—18.2 $\times$ 10^{-18} mole $bacterium^{-1}$ day^{-1}, which compares well with specific uptake rates by bacteria in groundwater, 0.2—3.9 $\times$ 10^{-18} mole $bacterium^{-1}$ day^{-1}, when incubated at 10 °C at 2 μm glucose (Smith et al. 1991). The higher specific uptake rates in Deer Creek all occurred in early spring, March to mid-June, and the values generally decreased significantly as the water temperature increased in June.

The particulate ATP ranged from <3.0 to 158 ng L^{-1} for the two years and epiflourescent bacterial counts ranged from 0.29 to 2.1 $\times$ 10^6 cells mL^{-1}. The lowest values occurred in March 1985 and the higher values occurred in the early spring period. In Deer Creek, planktonic bacteria may originate in the watershed or in the epilithon, or may be produced from *in situ* growth in the flowing stream water. The relative importance of these sources of bacteria may vary seasonally, and this variation could be an underlying influence on relationships between planktonic heterotrophic activity and environmental conditions.

The changes in microbial variables for the two-year study were compared with changes in discharge, DOC, and temperature (Table 1, Fig. 3). In 1984, maximum values of DOC and ATP concentrations, and enriched glucose uptake rates occurred on the first sampling date (May 23), which preceded the peak discharge by three weeks. The lowest glucose turnover time also occurred on this date. Concentrations of suspended organic carbon (SOC) were much lower than DOC concentrations, and varied only between 0.2 and 0.3 mg C/L while DOC concentrations were much more variable.

Water temperature increased gradually during snowmelt. A week after peak discharge, the water temperature was still only 4.5 °C. The maximum water temperature of 10.5 °C occurred on July 24 and the water temperature was 6—6.5 °C for most of the other sampling dates in July and August.

The stepwise linear multiple regression analysis was performed on the

Table 1. Selected data for hydrologic regime, dissolved organic carbon and microbial parameters in Deer Creek during spring snowmelt and summer 1984 and 1985 [Part. ATP = particulate adenosine triphosphate; DOC = dissolved organic carbon; — = no data; temp = temperature; glu-up = glucose uptake; T-time = glucose turnover time; D-cnts = direct bacterial counts].

Date	Discharge ($m^3 s^{-1}$)	Temp (°C)	DOC ($mgC L^{-1}$)	Glucose ($nmol L^{-1}$)	*In situ* glu-up ($nmol L^{-1} h^{-1}$)	Enriched glu-up ($nmol L^{-1} h^{-1}$)	T-time (h)	Part. ATP ($ng L^{-1}$)	D-cnts ($cells mL^{-1} \times 10^6$)
				1984					
5/23	0.24	0.5	5.2	—	—	2.9	40.2	158	—
5/29	0.36	—	4.2	—	—	1.6	105	85	—
6/4	0.42	4.0	3.1	—	—	0.28	262	50	—
6/14	1.53	4.5	4.1	—	—	1.2	223	67.8	—
6/19	1.11	4.5	3.1	83	0.35	0.58	236	50.5	—
6/26	0.83	5.5	3.0	—	—	0.93	227	33.3	—
7/5	0.72	6.0	2.4	111	0.43	0.33	260	62	—
7/11	0.68	6.0	2.6	—	—	1.2	218	59.5	—
7/17	0.40	6.5	2.3	—	—	0.2	300	32.7	—
7/24	0.30	10.5	1.9	—	—	1.04	168	46.3	—
7/30	0.47	8.0	2.6	<11	<0.05	1.15	227	63.6	—
8/9	0.35	6.0	2.3	39	—	—	—	42.5	—
8/21	0.26	6.5	2.7	—	—	—	214	67.4	—
9/7	0.32	3.5	2.5	111	0.10	0.27	1110	66.1	—

Table 1. (Continued)

					1985				
3/26	0.15	0.25	1.6	100	0.22	—	445	<3.0	0.29
4/16	0.15	0.25	2.9	—	—	—	57	140	1.4
4/23	0.15	0.25	2.5	—	—	—	213	38.4	0.8
4/30	0.15	0.25	2.3	100	0.54	—	185	22.6	0.9
5/7	0.15	0.25	4.5	<11	<0.19	—	58	107	2.3
5/14	0.15	0.25	2.1	56	0.35	—	160	36	1.1
5/21	0.23	2.0	3.4	44	0.28	—	157	21.4	1.6
5/28	0.35	3.0	3.8	97	0.97	—	100	49.5	2.1
6/4	0.40	4.0	3.3	333	2.06	—	163	36.2	0.86
6/11	1.11	5.0	3.4	83	0.62	—	136	45.0	1.1
6/25	0.62	5.0	2.6	39	0.16	—	243	16.8	0.57
7/9	0.35	8.0	2.0	83	0.30	—	279	56	0.67
7/23	0.30	12.5	2.4	28	0.13	—	217	29.5	0.94
8/6	0.26	8.5	2.0	39	0.16	—	240	17.1	1.1
9/26	0.23	1.0	1.2	211	0.22	—	984	102	0.52
10/22	0.23	0.5	1.4	67	0.19	—	340	7.4	0.41
2/20/86	0.15	0.5	1.7	111	0.23	—	479	21.3	0.40

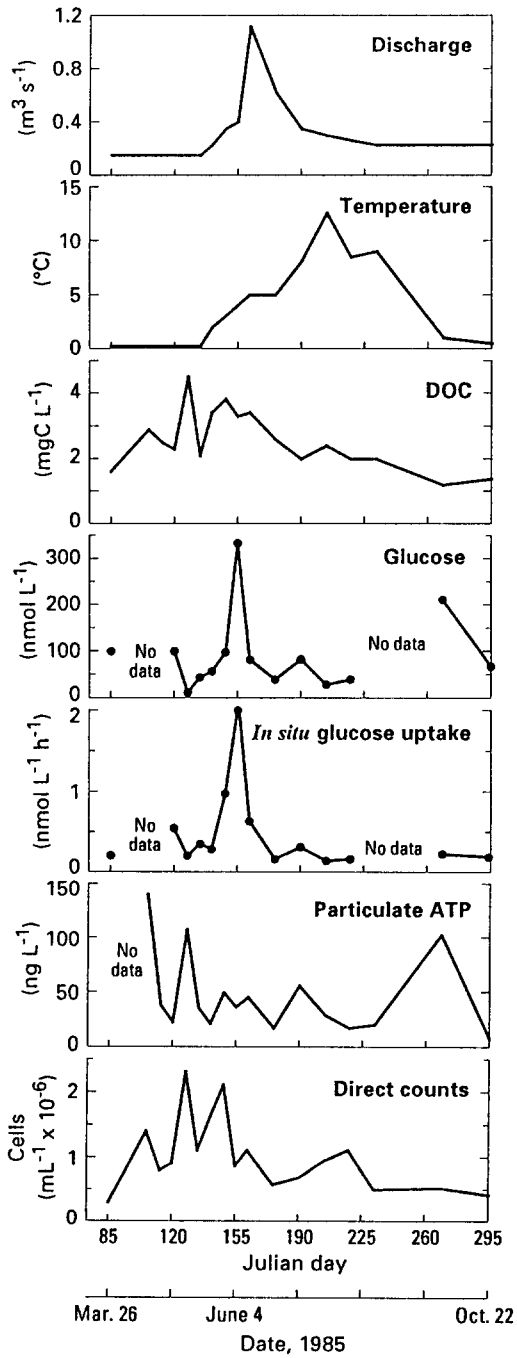

Fig. 3. Variation in Deer Creek during 1985 in discharge, stream temperature, DOC dissolved glucose, *in situ* glucose uptake rate, particulate ATP, and direct bacterial counts.

complete 1984 data set and for the period from May 23 to August 9, 1984, which encompasses the snowmelt dominated period. The regressions of glucose uptake on the individual variables were done using the first order rate constant (1/turnover time). The results for the snowmelt-only data are summarized in Table 2. DOC and discharge are identified as the most significant independent variables related to three microbial variables (ATP concentration, ^{3}H-glucose uptake rate constant, and enriched glucose uptake rate). Temperature was not related to the microbial variables. The microbial variables were positively correlated with DOC and negatively correlated with discharge, and a substantial percent of the variation was explained.

In 1985, sampling began in March, when the stream was still ice-covered. The DOC peak occurred on May 7, preceding peak discharge by a month. The maximum SOC (0.45 mg C/L) occurred with the maximum DOC (4.5 mg C/L) on May 7, and on other sampling dates SOC concentrations were similar to values measured in 1984. The maximum values of ATP, direct counts of bacteria, and the lowest glucose turnover time also occurred on May 7. However, the maximum glucose concentration and maximum glucose uptake rate occurred on June 4, three weeks after the DOC peak and one week before peak discharge. The lowest glucose turnover time occurred when the glucose concentration (pool size) was much lower than at other times.

Peak discharge in 1985 was less than peak discharge in 1984 and discharge decreased to lower values more rapidly in 1985 than in 1984. Correspondingly, in 1985 the water temperature increased more rapidly after peak discharge and a higher peak temperature was reached (12.5 °C in 1985 vs. 10.5 °C in 1984). The differences in the hydrologic regime reflect a lesser snowpack in 1985 than in 1984.

The data for the winter and snowmelt dominated period (until August 6, 1985) were analyzed using four dependent microbial variables (Table 2). Glucose concentration was included as an independent variable in the initial regression for glucose uptake. The results for the 1985 data were similar to the 1984 results in that (1) DOC concentration was positively correlated, with t-ratios greater than 2, with all of the dependent microbial variables except ATP, (2) all the microbial variables were negatively correlated with discharge, and (3) temperature was not related to any microbial variable. The differences between the two years were that (1) discharge had a t-ratio greater than 2 only in the regression for direct counts, and (2) the amount of the variance explained by the regressions for ATP and ^{3}H-glucose uptake rate constant was less for 1985 than for 1984. In the regression equations for ATP and ^{3}H-glucose uptake rate constant, the coefficients associated with DOC concentration and dis-

Table 2. Stepwise multiple linear regression analysis of relationship between microbial variables and environmental variables [DOC = dissolved organic carbon, mg C/L; D-cnts = direct bacterial counts, cells/mL × 10^6; Q = discharge, $m^3\ s^{-1}$; ATP = particulate adenosine triphosphate, ng/L; k = ^{3}H-glucose uptake rate constant × $10^{-3}\ h^{-1}$; v_{Glu} = glucose uptake rate, nmol $L^{-1}\ h^{-1}$; v_{en} = enriched glucose uptake rate, nmol $L^{-1}\ h^{-1}$; Glu = glucose, nmol L^{-1}; — not used as an independent variable].

	1984								
Variable	Regression coefficients				t-ratio				R^2
	Constant	DOC	Q	Glu	Constant	DOC	Q	Glu	
ATP	−11.7	30	−28.8	—	−0.64	5.55	−2.11	—	0.73
k	−4.6	5.1	−7.5	—	−1.29	5.08	−2.96	—	0.75
v_{en}	−0.5	0.61	−0.58	—	−0.89	3.86	−1.47	—	0.59
	1985								
ATP	−14.3	25.4	−36.7	—	−0.43	2.18	−1.02	—	0.19
k	−3.3	4.5	−5.7	—	−1.02	3.83	−1.58	—	0.50
v_{Glu}	−0.42	0.15	0.058	0.006	−2.6	2.78	−0.35	11.2	0.93
D-cnts	−0.38	0.62	−0.68	—	−1.4	6.30	−2.25	—	0.75

charge are close to those determined in the analysis of the 1984 data. Three of the four pairs of coefficients are within 20% or less and the coefficients for discharge in the ^{3}H-glucose uptake rate constant equations are within a factor of 2.

The weak correlation with ATP in 1985 may reflect sources of particulate ATP other than heterotrophic bacteria. Diatoms are dominant in the streamwater, with 31 different species identified during 1985. Algae released by grazing may be more significant during the low flows of 1985. This possibility is supported by the occurrence of maximum algal abundance on September 26, 1985, when the particulate ATP concentration was very high (102 ng/L) without an increase in bacterial abundance. On that date, the most abundant diatoms were *Fragilaria vaucheriae* (156 cells/mL), *Hannea arcus* (57 cells/mL) and *Achnanthes linearis* (27 cells/mL).

Diel variations in heterotrophic activity

There are three potential sources of heterotrophic bacteria in Deer Creek; the watershed, the epilithon and *in situ* growth. Benthic invertebrate drift and associated grazing activity typically increases at night and could cause

the release from the epilithon of fine particulate organic material, including bacteria and algae (Iversen et al. 1982). Short term fluctuations in microbial activity were studied on two occasions in the autumn of 1984, when the low flows and full development of the epilithon should have maximized the significance of an epilithic bacterial source. The sampling on September 9 was begun in the early evening (6:00 P.M.) after a major afternoon thunderstorm, and was continued until the following afternoon at 2:45 P.M. During the November 7—9 study, heavy snowfall began at 11:00 A.M. on November 8 and continued throughout the last 24 h of the study. DOC concentrations were between 2.0 and 2.2 mgC L^{-1} during both studies and discharge was 0.26 m^3 s^{-1} for the September study and 0.23 m^3 s^{-1} for the November study. Glucose concentration was determined on September 7, two days before the study, and once during November 7—9 and was 111 nM on both dates. This value was used to calculate *in situ* glucose uptake rate from the tritiated glucose uptake rate. The temperature, *in situ* glucose uptake rate and drifting benthic invertebrate results are presented in Fig. 4. Chlorophyll *a* concentrations remained below the detection limit of 1 μg/L.

Heterotrophic activity fluctuated over short time scales in both studies. The general pattern was that glucose uptake was depressed during daylight and elevated at night and during the snowstorm. The diel range in heterotrophic activity was less than in the seasonal data. Stream temperature appeared to have no influence on heterotrophic activity in either

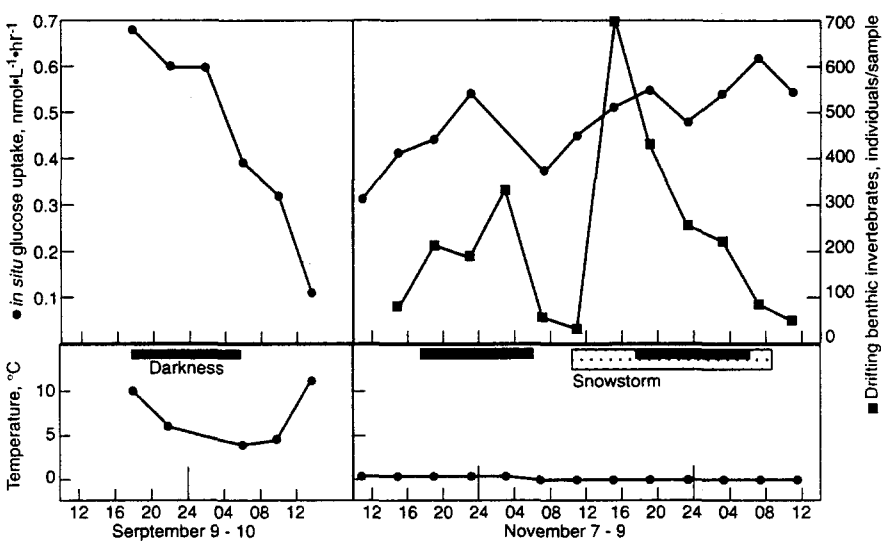

Fig. 4. Diel variation in temperature, glucose uptake rate and benthic invertebrate drift.

study. In November, increased heterotrophic activity appeared to be related to increased benthic invertebrate drift activity and associated grazing (Fig. 4). The most abundant benthic invertebrates were mayflies (*Baetis bicaudata* and *Cinygmula sp.*) and stoneflies (*Taenionema nigripene* and *Zapada haysii*) which increased at nightfall and during the snowstorm (Fig. 4 and Miller, unpublished data) in both Deer Creek and the adjacent Snake River. The dramatic burst in drift activity with the onset of the snowstorm involved most species, including the flies and beetles. In addition to benthic invertebrate activity, direct photo-inhibition by sunlight may contribute to this pattern. The water was shallow in the autumn, ranging from 5 cm in ripples to 40 cm in pools, and would not afford much protection from incident radiation. In summary, epilithic bacteria may be an important source of planktonic bacteria in autumn, and grazing by benthic invertebrates could control this source. However, the epilithon is not likely to be an important source of bacteria under high discharge during snowmelt or in early spring under snow cover when the epilithon is less developed (Table 1, March 26, 1985).

Influences of the snowpack

Snowpack in the riparian zone near the study site was sampled in mid-winter and during early snowmelt to evaluate organic substrates which could support heterotrophic activity in the spring (Table 3). The winter sample contained low DOC and mid-range glucose concentrations (0.9 mg C/L and 94 nM, respectively) and had a low heterotrophic activity when the snow was melted. The spring sample, which had a coating of fine dirt, had DOC concentrations comparable to those in the stream. The glucose concentration was higher than those found in the stream at any time of the year (Table 1), and the glucose uptake rate was greater than rates

Table 3. Microbial populations and heterotrophic activity in snow samples collected adjacent to the Deer Creek study site on two occasions.

Sample date	DOC (mg C L^{-1})	Glucose[1] (nmol L^{-1})	Heterotrophic activity	
			Glucose turnover time (h)	Glucose uptake rate (nmol L^{-1} h^{-1})
5/5/85	2.5	444 ± 78	42	10.6
2/20/86	0.9	94 ± 23	9350	0.01

[1] Mean and standard deviation of duplicate analyses are reported.

measured in the stream. The accumulated windblown dirt may be a source of soluble organic material, nutrients and bacteria. These results indicate a potential for the snowmelt to vary in its content of soluble organic material and heterotrophic bacteria.

Leaching of riparian zone litterfall

The leaves fall from the willow bushes in the riparian zone about the same time as the snowpack begins to accumulate. Fallen leaves and scenescent sedges and other vegetation represent potential sources of soluble organic carbon. Changes in willow-leaf packs placed in the riparian zone were followed through the winter snowfalls and spring snowmelt (Fig. 5). Weight loss under snow was fairly linear with time, and reached about 30% by the end of snowmelt.

The DOC concentration of the leaf leachates was greatest on the first retrieval date, after 20 days, and decreased greatly (e.g. 26%) by the next retrieval date. From then on, the leachate DOC concentrations decreased slightly (e.g. 1—5%), and DOC could still be leached from the leaves after the snowpack melted. The percentage of the leachate DOC accounted for by hydrophobic neutrals and acids and by fulvic acid alone changed throughout the winter. The hydrophobic neutral and acid fraction increased to 44% of the DOC and then decreased a few weeks before the snowpack had completely melted. On the last retrieval date this fraction accounted for 29% of the DOC. Fulvic acid was less variable, ranging from 9 to 14% of the DOC.

In addition to these hydrophobic fractions, soluble organic material in the leafpacks may include carbohydrates (Hurst et al. 1985). A subset of the leafpacks were analyzed for dissolved glucose concentrations in the leachates, which ranged from 4—20% of the DOC. Compared to the stream, glucose is a much more significant DOC fraction in the leachates.

The organic material lost from the leaves by leaching under the snow may be stored in the snow and ice at the interface of the snowpack and the litter layer, in the litterfall, or in the upper soil horizons. When the snow and ice melts, this soluble organic material becomes dissolved in the interstitial water, which is then flushed into the stream as snowmelt progresses. Thus the leaching of leaves in the riparian zone potentially contributes to the increase in stream DOC concentrations at the beginning of snowmelt.

The DOC distribution among different chemical fractions is expected to change as the DOC is transported to the stream. Fulvic acid may increase as a percentage because it is less microbially labile than other DOC fractions (Moran & Hodson 1990). Glucose and other labile sub-

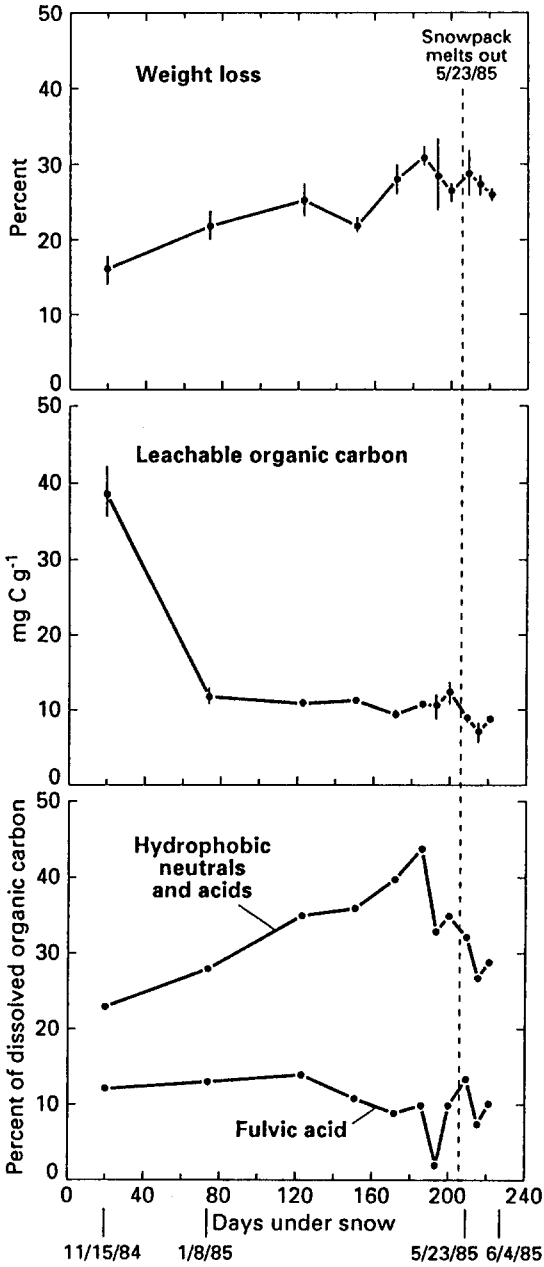

Fig. 5. Percent weight loss of willow leaf packs under snow pack during the winter and spring; concentration of leachable dissolved organic carbon (DOC) per gram dry weight of leafpack; and percent of leachable DOC as hydrophobic acids and neutrals and as fulvic acid.

strates may be assimilated by microbial populations in the upper soil layers and in the litter layer, or in the substream zone. Vervier & Naiman (1992) have shown that DOC uptake can occur during substream flow through a gravel bar. Differential uptake during transport can explain fulvic acid being a greater proportion of the DOC in the streamwater than in the leachates, and glucose being a lesser proportion.

Conclusions

During snowmelt, heterotrophic activity increased in response to increasing DOC concentrations, despite the low temperatures of the streamwater. The measures of bacterial biomass and activity varied with DOC concentration during snowmelt. This relationship reflects the response of the bacterial populations in the stream to increased concentrations of more labile DOC that probably occurs with the increases in total DOC.

Microbial activity was negatively correlated with discharge during snowmelt. There are several possible explanations. As snowmelt progresses, streamflow is generated from snowpack higher in the watershed which may carry less bacteria and DOC. Also, the distance traveled from the melting snowpack to the sampling site increases. During initial snowmelt, DOC-rich meltwater coming into the stream from riparian zones may travel a short distance through shallow soil horizons with little enroute uptake of labile substrates. Later on, meltwater may travel longer distances through shallow soil horizons, with a greater uptake of the most labile DOC components by soil microorganisms. Furthermore, the nutritive quality of the DOC may decrease as it is transported downstream as a result of instream heterotrophic uptake (Bott et al. 1984). This scenario, however, would not explain why the glucose concentration peaked after the DOC concentration in 1985.

An unanticipated conclusion from this study is that the low temperature of the streamwater during snowmelt or at night did not have a significant limiting effects on planktonic heterotrophic activity. Instead, the concentration of dissolved organic material and some aspect of the hydrologic flowpath bringing the water into the stream have significant effects over seasonal time scales, and benthic invertebrate activity or photoinhibition may have effects over diel time scales. The conclusions regarding the hydrologic flowpath are supported by the demonstration that the riparian zone litterfall can be a source of soluble organic carbon including labile substrates which may be mobilized into the stream during snowmelt.

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References

Aiken GR, McKnight DM, Thorn KA & Thurman EM (1992) Isolation of hydrophilic organic acids from water using macroporous resins. Org. Geochem. 18(4): 567—573

Baker BL, Ladd TI, Telang SA, Costerton JW & Hodgson GW (1982) The biogeochemistry of streams: The Red Deer River. Alberta Research Management Division Report, RMD 82-154, 221 pp

Baker JH (1986) Relationship between microbial activity of stream sediments determined by three different methods, and abiotic variables. Microb. Ecol. 12: 193—203

Baron J, McKnight DM & Denning AS (1991) Sources of dissolved and suspended organic material in Loch Vale Watershed, Rocky Mountain National Park, Colorado, USA. Biogeochem. 15: 89—110

Bott TL, Kaplan LA & Kuserk FT (1984) Benthic bacterial biomass supported by stream-water dissolved organic matter. Microb. Ecol. 10: 335—344

Bulleid NC (1978) An improved method for the extraction of adenosine triphosphate from marine sediments. Limnol. Oceanog. 23: 174—178

Cavari BZ & Phelps G (1977) Sensitive enzymatic assay for glucose determination in natural waters. Appl. Environ. Microbiol. 33: 1237—1243

Denning AS, Baron J, Mast MA & Arthur M (1991) Hydrologic pathways and chemical composition of runoff during snowmelt in Loch Vale Watershed, Rocky Mountain National Park, Colorado, USA. Water, Air Soil Poll. 59: 107—123

Easthouse KB, Mulder J & Christophersen N (1992) Dissolved organic carbon fractions in soil and stream water during variable hydrological conditions at Birkenes, Southern Norway, Water Resour. Res. 28(6): 1585—1596

Gocke K, Dawson R & Liebezeit G (1981) Availability of dissolved free glucose to heterotrophic microorganisms. Mar. Biol. 62: 209—216

Goulder R (1984) Downstream increase in the abundance and heterotrophic activity of suspended bacteria in an intermittent calcareous headstream. Freshwater Biol. 14: 611—619

Hobbie JE (1967) Glucose and acetate turnover in freshwater: Concentrations and turnover rates: In: Chemical Environment in the Aquatic Habitat, Proceedings of an I.B.P. symposium. Amsterdam, 10—16 October, 1966

Hornberger GM, Bencala KE & McKnight DM (submitted) Hydrologic controls on the temporal variation of dissolved organic carbon in the Snake River near Montezuma, Colorado. Biogeochemistry

Hurst JL, Pugh GJF & Walton DWH (1985) The effects of freeze-thaw cycles and leaching on the loss of soluble carbohydrates from leaf material on two subantarctic plants. Polar Biol. 4: 27—31

Iversen TM, Thorup J & Skriver J (1982) Inputs and transformation of allochthonous particulate organic matter in a headwater stream. Holarctic Ecol. 5: 10—19

Lewis WM Jr & Grant MC (1979) Relationships between flushing and yield of dissolved substances from a mountain watershed. Soil Sci. 128: 353—363

McKnight DM, Bencala KE, Zellweger GW, Aiken GR, Feder GL & Thorn KA (1992) Sorption of dissolved organic carbon (including fulvic and hydrophilic acids) by hydrous aluminium and iron oxides: A study of processes occurring at the confluence of Deer Creek with the Snake River, Summit County, Colorado, Environ. Sci. Technol. 26: 1388—1396

McKnight DM & Bencala KE (1990) The chemistry of iron, aluminium, and dissolved organic material in three acidic, metal-enriched, mountain streams, as controlled by watershed and in-stream processes. Water Resour. Res. 26: 3087—3100

McKnight DM & Feder GL (1984) The ecological effect of acid conditions and precipitation of hydrous metal oxides in a Rocky Mountain stream. Hydrobiol. 119: 129—138

Meyer JL (1990) Production and utilization of dissolved organic carbon in riverine ecosystems. In: Perdue EM & Gjessing ET (Eds) Organic Acids in Aquatic Ecosystems (pp 281—299). John Wiley and Sons, Ltd

Minitab, Inc (1988) Minitab Reference Manual

Mopper K, Dawson R, Liebezeit G & Ittekot V (1980) The monosaccharide spectra of natural waters. Mar. Chem. 10: 55—66

Moran MA & Hodson RE (1990) Bacterial production on humic and nonhumic components of dissolved organic carbon. Limnol. & Oceanog. 35: 1744—1756

Murray RE & Hodson RE (1984) Microbial biomass and utilization of dissolved organic matter in the Okefenokee Swamp ecosystem. Appl. Environ. Microbiol. 47: 685—692

Smith RL, Harvey RW & LeBlanc DR (1991) Importance of closely spaced vertical sampling in delineating chemical and microbiological gradients in groundwater studies. J. Cont. Hydrol. 7: 285—300

Theobald PK, Lakin HW & Hawkins DB (1963) The precipitation of aluminum, iron, and manganese at the junction of Deer Creek with the Snake River in Summit County, Colorado. Geochim. Cosmochim. Acta 27: 121—132

Thurman EM (1985) Organic Geochemistry of Natural Waters. Junk Publishers, 497 p

Thurman EM & Malcolm RL (1981) Preparative isolation of aquatic humic substances. Environ. Sci. Technol. 15: 463—466

Vaccaro RF, Hicks SE, Jannasch HW & Carey FG (1968) The occurrence and role of glucose in seawater. Limnol. Oceanog. 13: 356—360

Vervier Phillipe & Naiman Robert J (1992) Spatial and temporal fluctuations of dissolved organic carbon in sursurface flow of the Stillaguamish River (Washington, USA). Arch. Hydrobiol. 123(4): 401—422

Wright RT & Hobbie JE (1966) Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecol. 47: 447—464