

Changes in composition and reactivity of allochthonous DOM in a prairie saline lake

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Abstract

Inland saline lakes in semiarid regions of the Canadian prairies contain some of the highest known concentrations of dissolved organic carbon (DOC). This dissolved organic matter (DOM) represents a potentially important carbon and energy source for aquatic bacteria. Redberry Lake, an oligotrophic saline lake in central Saskatchewan, is located in a hydrologically closed basin and has high levels of DOC (seasonal mean 35 mg L⁻¹). Despite such high DOC concentrations, lake water is clear. Lake DOM is predominantly allochthonous, and enters the lake via the major inflow, Oscar Creek. Despite its origin, this DOM is compositionally much different than its creek counterpart. Approximately 73% of total lake DOM is low in molecular weight (<1000 D). XAD-8 isolated hydrophobic organic acids (HPOA) are low in aromaticity, have high C:N ratios and a certain percentage are old (~700 years). In comparison, creek water, despite having lower DOC concentrations than the lake (14.9 mg L⁻¹) is highly colored. Fifty-five per cent of this DOC is low in molecular weight and isolated DOM has higher aromaticity and lower C:N ratio than lake DOM. As a result of these changes in DOM, ultraviolet light penetrates much deeper into lake water as compared to the creek. Photolysis experiments revealed that DOM in Oscar Creek and Redberry Lake is photoreactive. Changes in lake DOM are not only linked to location within a hydrologically closed basin and photochemistry, but provide the explanation for the optically different character of DOC in this geographical region. Changes in lake DOM have had an effect at the microbial level as well. Little of the lake DOM appears available for bacterial growth as a result of these photochemical changes. Creek DOM, having a shorter residence time, does not appear to have been as photochemically changed and consequently is more available for bacterial growth.

Aquatic systems with high concentrations of dissolved organic carbon (DOC) are characteristically tea-colored (Thurman 1985). Moreover, as DOC concentration increases, so does color (Tranvik 1990). Inland saline lakes in semiarid regions of the Canadian prairies are different. Although they contain some of the highest known concentrations of DOC, it is generally less colored than that originating in humid regions. As salinity increases, so too does DOC concentration (Curtis and Adams 1995).

Redberry Lake is an inland saline lake located in a hydrologically closed basin in south central Saskatchewan, Canada. Although DOC concentrations in the lake are high (seasonal mean 35 mg L⁻¹) the lake water is not colored (Arts et al. 2000). In direct contrast to the lake, water in the major inflow is highly colored. Obviously DOM which has

travelled from the inflow into the lake has undergone major changes. An earlier study concluded that little of the DOM in this lake is available for bacterial uptake (Waiser and Robarts 1997) and this fact might be directly linked to changes in DOM character.

The goal of this study was to identify and characterize these changes and establish how they might affect optical characteristics of DOC and microbial carbon cycling. This problem was approached from a biological and chemical perspective. At the biological level, we wanted to quantify and compare the amount of DOM in Redberry Lake and the major inflows which was available for bacterial uptake. Because of the clear nature of the lake water, we were also interested in the possibility that photolysis of DOM in lake water might be releasing labile carbon substrates suitable for bacterial uptake and in so doing change the constitution of the DOM. At the chemical level, we wanted to characterize the DOM from both lake and inflow. DOM was isolated from both lake and inflow on XAD-8 resins and subsequently analysed for age (¹⁴C dating), source (δ^{13} C signature), composition (¹³C nuclear magnetic resonance spectroscopy), and C:N ratios. In addition, electrospray mass spectrometry and ultrafiltration were utilized to establish the molecular weight spectrum for DOM and a scanning spectrofluorometer to establish DOM fluorescence. As well, a scanning spectroradiometer was used to calculate diffuse attenuation coefficients for ultraviolet-A (UV-A), UV-B and photosynthetically active radiation (PAR) radiation in both the lake and the major inflow, Oscar Creek. Not only would these methods allow us to identify changes in DOM character, they would also provide some fundamental insights into the way in which carbon is cycled in this prairie saline lake.

Materials and methods

Study site—Redberry Lake (Z_{\max} = 16 m, surface area 45 km²) is an oligotrophic (mean summer Chl *a* 0.7 μ g L⁻¹;

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mean summer secchi disk 5.0 m) (Waiser and Roberts 1995) alkaline saline lake situated in a flat-bottomed glacial kettle on a glacial lacustrine alluvial plain in south-central Saskatchewan (52°43'N, 107°09'W). The lake basin is hydrologically closed (endorheic); there are three freshwater inflows (Oscar, Marshy, and Trout Pond creeks) but no outflow (Van Stempvoort et al. 1993). At springmelt, Oscar Creek contributes close to 90% of the total annual streamflow (Van Stempvoort et al. 1993). All inflowing water is highly colored. There are no significant aquifers for the Redberry Lake area and mass balance calculations indicate no significant input of groundwater to the lake (G. Van der Kamp, N.W.R.I. pers. comm.).

Because of the endorheic nature of the drainage basin and its location within a semiarid climatic zone where evaporation exceeds precipitation, Redberry Lake has steadily decreased in volume by approximately 50–60% since 1918, with concomitant increases in salinity (Van Stempvoort et al. 1993). The mean residence time of the water in the lake is 20 years. In this type of basin, residence time is not associated with flushing rate, but with evaporation. Lake salinity (total dissolved solids, 20.9 g L⁻¹) is dominated by magnesium (68.1%), and sodium (29.2%) cations, and sulfate (93.7%) anions (Arts et al. 1993). Although levels of soluble reactive phosphorus in the lake are high (summer mean 16 µg L⁻¹), little is available for microbial uptake, and consequently the microbial population shows strong signs of P-limitation (Waiser and Roberts 1995). Concentrations of NO₂ + NO₃-N are usually <2.0 µg L⁻¹ while NH₃-N concentrations are mostly <30 µg L⁻¹ during the ice-free season (Roberts et al. 1992). As mentioned, although DOC concentration averages 35 mg L⁻¹ (seasonal mean) the lake water is not colored. Mean annual euphotic zone depth (Z_{eu}) for this lake is 15.2 m (Roberts et al. 1992).

DOM availability experiments—Several experiments were carried out in July of 1996 to determine the amount of biologically available carbon in Redberry Lake and its three major inflows: Marshy, Oscar, and Trout Pond creeks. Water samples for these experiments were collected, placed in acid-washed carboys, and transported on ice in the dark to the laboratory. After prescreening through 153 µm Nitex mesh to remove large zooplankters, water was filter sterilized through 0.2 µm cellulose nitrate filters (previously rinsed with copious amounts of Milli-Q water), 150 ml placed into each of 6 autoclaved flasks, and a bacterial inoculum (10 ml) from the source water was added (Servais et al. 1989). The bacterial inoculum consisted of screened lake water which had been passed through combusted GF/C filters (nominal pore-size 1.2 µm). Although we did not test for efficiency of bacterivore removal from the inoculum, heterotrophic protists in the size class 2–20 µm are assumed to be the principal predators for bacteria (Gasol et al. 1995) and therefore the GF/C filter should have removed bacterivores. However, we do acknowledge, that these filters do not necessarily remove all bacterivores and that a small number could have remained in the inoculum. Although some have argued strongly that in such studies, the use of a “defined” microbial inocula is warranted in order to provide an absolute measure of DOC quality (Leff and Meyer 1991), the use

of a native assemblage in this study was justified. As we were working with fresh and saline water, it would have been impossible to develop a defined inocula which would grow in both systems.

Three of the flasks received sterile additions of 1,700 µg L⁻¹ nitrogen (as NaNO₃) and 105 µg L⁻¹ phosphorus (as KH₂PO₄) at 3-d intervals. Flasks were well shaken after nutrient additions. All flasks were incubated at 20°C in the dark for 4 weeks (Servais et al. 1989) and hand shaken twice per day. According to Servais et al. (1989) the time required for maximum DOC decrease in biologically available carbon experiments using natural waters is between 3–4 weeks. As well, having run the nutrient enrichment experiment (*see below*) before the bioavailability experiments, we knew that, at least for the lake water, DOC appeared to be quite refractory and therefore a short incubation time might not show any utilization of DOC. However, it is acknowledged that the long incubation time was probably more suitable for Redberry Lake bacteria (long water residence time) than for the creek (short residence time) (c.f., Leff and Meyer 1991).

At the beginning and end of the experiments, samples were withdrawn for bacterial enumeration and DOC concentration. Bacterial samples were preserved (Pomroy 1984) and subsequently counted using 4',6-diamidino-2-phenylindole (DAPI) and epifluorescent microscopy (Tumber et al. 1993), while DOC was analysed according to methods in Environment Canada (1992). The amount of biologically available carbon in each of the experiments was calculated as the difference between the means of the DOC concentrations at the beginning and end of the experiment (Servais et al. 1989). As well, the amount of bacterial carbon (in µg) produced per mg of ambient DOC (also a measure of biological availability) was estimated by first subtracting initial bacterial numbers from final numbers, multiplying this number by a carbon conversion factor of 16.28 fg C cell⁻¹ (Roberts et al. 1999) and then dividing the amount of bacterial carbon produced (in µg) by the DOC concentration (in mg) at the beginning of the experiment (Leff and Meyer 1991). Because it is likely that bacterivores were present in the incubation fluid, the measures of biological carbon availability based on bacterial carbon accumulations should be viewed as underestimates.

For all experiments, a paired sample *t*-test (SIGMASTAT) was used to test for differences between DOC concentrations and bacterial numbers at beginning and end of experiment.

Nutrient enrichment experiments—A short term microcosm experiment was also used to examine bacterial utilization of DOM. Microcosms consisted of a volume of filtered lake water (*see below*) to which labile organic substrates and nutrients (singly and in combination) were added. The microcosms were then monitored over time for changes in bacterial growth (bacterial numbers), metabolism (¹⁴C-glucose uptake), and production (rate of incorporation of ³H-thymidine into bacterial DNA).

Water for microcosms was collected from the epilimnion of Redberry Lake in August 1993, transported and prescreened as noted above. After prescreening, the water was passed through Whatman 934-AH glass fibre filters (nominal pore-size 1.5 µm). Exclusion of phytoplankton in this man-

ner ensured a direct bacterial growth response to carbon and nutrient amendments (Coveney and Wetzel 1992). Earlier experiments revealed that filtration through the 934-AH filter removed 77% of the Chl *a* while allowing 74% of the bacteria to pass through (Waiser and Robarts 1997). All flasks were subsequently placed in a dark environmental chamber at lake temperature (18°C) to acclimatize overnight.

The next day, water in the flasks was treated in the following manner: a control with no amendments; an organic substrate treatment (AA) in which 200 $\mu\text{g L}^{-1}$ each of serine, glycine, glycolate, and glycyglycine were added (Coveney and Wetzel 1992); a treatment in which nitrogen and phosphorus (N+P) were added (105 $\mu\text{g L}^{-1}$ as P; 1,700 $\mu\text{g L}^{-1}$ as N), and finally, a treatment in which organic substrates, N and P were added together (N+P+AA). Amino acids were chosen as an organic substrate because in addition to being well known organic C sources for aquatic bacteria, they would not cause isotope dilution (as glucose would) during ^{14}C -glucose uptake estimates. Treatments were run in triplicate (12 flasks total). Following treatment, flasks were well mixed, placed on a shaker table in an environmental chamber, and incubated in the dark at lake temperature (18°C).

At intervals of 0 (start value), 3, 27, 51, and 75 hours, samples for bacterial numbers (10 ml) were withdrawn, preserved and counted as described before. Two samples (one live and one control) of 10 ml each were also withdrawn from each microcosm flask for bacterial metabolism and production measurements. Samples for bacterial metabolism estimates (including respiration), were amended with uniformly labeled high specific activity ^{14}C -glucose (>230 mCi mmol^{-1}) at a saturating concentration of 125 $\mu\text{g L}^{-1}$. After 1.5 h incubation, samples were processed and filtered according to the method of Hobbie and Crawford (1969) and then radioassayed with a liquid scintillation spectrometer. Rates of ^{14}C -glucose uptake (v) were calculated according to the specific activity equation of Wright (1978) for substrate concentrations >100 $\mu\text{g L}^{-1}$.

Bacterial production was estimated by the rate of [methyl- ^3H] thymidine (TdR) incorporation into bacterial DNA (Robarts and Wicks 1989). Reported time zero bacterial numbers, metabolism, and production rates represent mean values derived from analysis of prescreened water from the three carboys.

A two-way ANOVA on bacterial production rates, glucose metabolism rates, and numbers from day three of the experiment (when maximum bacterial numbers and rates of TdR incorporation and glucose uptake were seen) followed by a post hoc Tukey's test was used to test for differences in treatments (SIGMASTAT). The significance level for testing was $P < 0.001$.

Photolysis experiments—Epilimnetic water from Redberry Lake and Oscar Creek was collected in June 1999, prescreened, and transported as noted above. Subsequently, water was tangentially filtered using a Centramate system equipped with 20.0 and 1.0 μm prefilters and 0.1 μm pore-size polyethersulfone filter pack (Pall Filtron). Bacterial concentrate (from the retentate) and sterile filtrate thus generated were stored in the dark in sterile glass containers at 4°C until

use. Subsamples were removed from both unfiltered and 0.1 μm filtered water to estimate sterilization efficiency of the 0.1 μm filter pack.

The next day, 2 L of sterile filtrate were placed into each of 6–3 L shallow baking dishes (12 L total). Samples were withdrawn from each dish and preserved as above for bacterial counts. All baking dishes were acid-washed and autoclaved prior to use. Baking dishes were then covered with foil (control, no light treatment) or polyvinylidene (Saran, Dow Chemical, total light treatment). Saran is 80–90% transparent throughout the UV-A and UV-B regions and transmits 90% of PAR between 400 and 700 nm (Bothwell et al. 1993). All treatments were run in triplicate. Glass baking dishes were randomly placed in two flow-through water baths located on the roof of the National Hydrology Research Centre (NHRC, Saskatoon) and exposed to natural radiation for 5 d (exposure period). During incubation, automated scans of solar radiation were made between 0900 h and 1600 h every 30 min at 2 nm intervals from 280 to 700 nm using a scanning spectroradiometer (Optronics Laboratories, Model OL-754).

At the end of the exposure period samples were removed from each baking dish and preserved as above for bacterial counts. This was done to ensure that sterility of the water had been maintained over the 5-d incubation period. For the subsequent bacterial growth phase, two 500 ml aliquots of water were removed from each baking dish and placed in 2 autoclaved and acid-washed 2 L wide-mouth flasks. To each 500 ml, a 100 ml inocula of the bacterial concentrate (from prior tangential filtration) was added bringing bacterial numbers to approximately ambient concentration. In addition, nitrogen and phosphorus, at concentrations previously noted, were added to one of the flasks from each of the dishes. Therefore, for each exposure treatment (three baking dishes) there were six flasks; three of which received nutrients and three which did not. All flasks were stoppered with autoclaved styrofoam plugs and placed on a shaker table in an environmental chamber in the dark at 20°C for three days.

At timed intervals of 2, 24, and 48 hours, two 10 ml samples (one live and one killed control) were withdrawn from each flask, amended with TdR, extracted and counted as above. In addition, a 10 ml volume of water was also withdrawn for bacterial numbers, preserved and counted as previously noted.

By day 3 of the bacterial growth phase, bacterial numbers and rates of TdR incorporation declined in all of the treatments (data not shown), and therefore the experiment was concluded. A *t*-test (SIGMASTAT) was used to test for differences in TdR incorporation and bacterial numbers for light and dark treatments.

DOM characterization—Samples of Redberry Lake epilimnetic and Oscar Creek water were collected in July 1998 in clean Nalgene carboys and transported back to the lab for size fractionation of DOM. All water was prescreened, as noted earlier, and then filtered through combusted GF/C and then GF/F filters. Aliquots of filtrate were ultrafiltered using a Centramate polyethersulfone tangential flow cartridge (Pall Filtron) with a molecular weight cutoff of 1,000 Daltons according to the method outlined in Curtis and Adams

Table 1. Summary of results from carbon availability experiments.

Site	Treatment	Increase in bacteria (10 ⁶ ml ⁻¹)	Decrease in DOC (mg L ⁻¹)	% DOC available	Bacterial C/initial ambient DOC (μg mg ⁻¹)
Redberry Lake	No N + P	none	none	ND	ND
	+N + P	2.1*	1.7	ND	0.96
Oscar Creek	No N + P	2.9*	1.6*	12.5	3.8
	+N + P	4.1*	1.9*	14.8	5.2
Marshy Creek	No N + P	7.4*	1.2*	9.2	9.3
	+N + P	6.9*	1.7*	13.2	8.7
Trout Pond Creek	No N + P	2.4*	1.2*	14.1	4.4
	+N + P	4.3*	1.6*	19.5	8.4

ND, not detected.

* Significant at $P < 0.05$.

(1995). Volumes of GF/F and 1,000 Dalton filtrate were subsequently analysed for DOC as noted above.

In August 1999, samples were collected from the lake and creek, filtered through GF/F filters and analysed for DOC fluorescence. Peak DOC fluorescence was measured at 370 nm excitation and 424 nm emission wavelengths using optically clear quartz cuvettes and a Shimadzu RF-1501 scanning spectrofluorometer equipped with a xenon lamp (Donahue et al. 1998). DOC fluorescence values were then normalized to DOC concentration and compared. As well, fluorescence was measured at emission wavelengths of 450 and 500 nm (370 nm excitation) and the ratio 450:500, as an indication of DOC source, was calculated (Donahue et al. 1998).

Twenty liter samples of Redberry Lake epilimnetic water and Oscar Creek water were collected in July 1998, placed in 25-liter glass carboys (previously acid washed and rinsed thoroughly with Milli-Q water), and transported back to the laboratory for subsequent extraction of DOM using XAD-8 resins. For both sites, water was sequentially filtered through combusted GF/C and GF/F filters (nominal pore-size 0.7 μm) the same day and some of the filtrate analysed for DOC. The remaining filtrate was acidified to pH 2 using 1 N HCl and then passed through a column filled with XAD-8 ion exchange resin. Some of the sample which passed through the resin bed was collected and analysed for DOC. The hydrophobic DOM fraction was eluted from the resin using 0.1 N NaOH (Thurman and Malcolm 1981) and will subsequently be referred to as hydrophobic organic acids (HPOA). The eluant was placed in acid-washed 1,000 ml Nalgene wide-mouth bottles, frozen at -40°C, and then freeze-dried to powder. In addition, a Redberry Lake whole water sample was filtered and freeze dried as above.

All freeze-dried samples were combusted at 850°C using the Dumas technique and the resulting CO₂ trapped and purified cryogenically for subsequent ¹⁴C and δ¹³C analysis (McGaw et al. 1988). Analyses of δ¹³C were done on a Fisons VG optima isotope ratio mass spectrometer at the National Hydrology Research (NHRC) Center and reported using the δ notation relative to the international Pee Dee Belemnite (PDB) standard where

$$\delta^{13}\text{C} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

and R is the ¹³C/¹²C ratio in the sample or the standard.

Radiocarbon analyses were done by tandem accelerator mass spectrometry at the Univ. of Toronto. The results were expressed in relation to the ¹⁴C/¹²C ratio in an international standard (oxalic acid) where the ¹⁴C concentration in bulk carbon of the standard is defined as 100% pmC (post modern carbon).

Some of the freeze-dried powder was also submitted to the United States Geological Survey lab in Boulder, Colorado and to the National Research Council Laboratory in Saskatoon for [¹³C] nuclear magnetic resonance (NMR) spectroscopy analysis according to the method of McKnight et al. (1991). Solid state NMR spectra were divided into specific chemical shift regions according to organic carbon functional groups present in the freeze-dried sample. The region between 0–60 ppm (AL-I) represents aliphatic carbons and includes both lipids and fatty acids. Carbohydrates dominate the region between 60–110 ppm and consist of two fractions; AL-II (60–90) and AL-III (90–110). The region between 110–160 ppm is representative of aromatic carbon (AR), including the phenolic region between 140–160 ppm. Carboxylic carbon (C-I) dominates the region from 160–190 while carbonyl signals (C-II, ketones and aldehydes) are present in the region from 190–210 ppm (McKnight et al. 1994; Clair et al. 1996). A CHN analyser was used to determine the carbon and nitrogen content of isolated DOM according to methods in Environment Canada (1992).

A portion of the freeze-dried eluant was also analysed by electrospray mass spectrometry in the negative ion mode using a Fisons Autospec Q mass spectrometer. A loop injection of the sample (no analytical separation of compounds) directly into the electrospray interface via an high performance liquid chromatography (HPLC) pump was made to determine the approximate average mass of the components contained in the eluant. Electrospray is a soft ionization technique which theoretically should cause little fragmentation of the sample (Voyksner 1994).

Underwater light scans—Underwater penetration of UV-B, UV-A, and PAR in Oscar Creek and Redberry Lake was

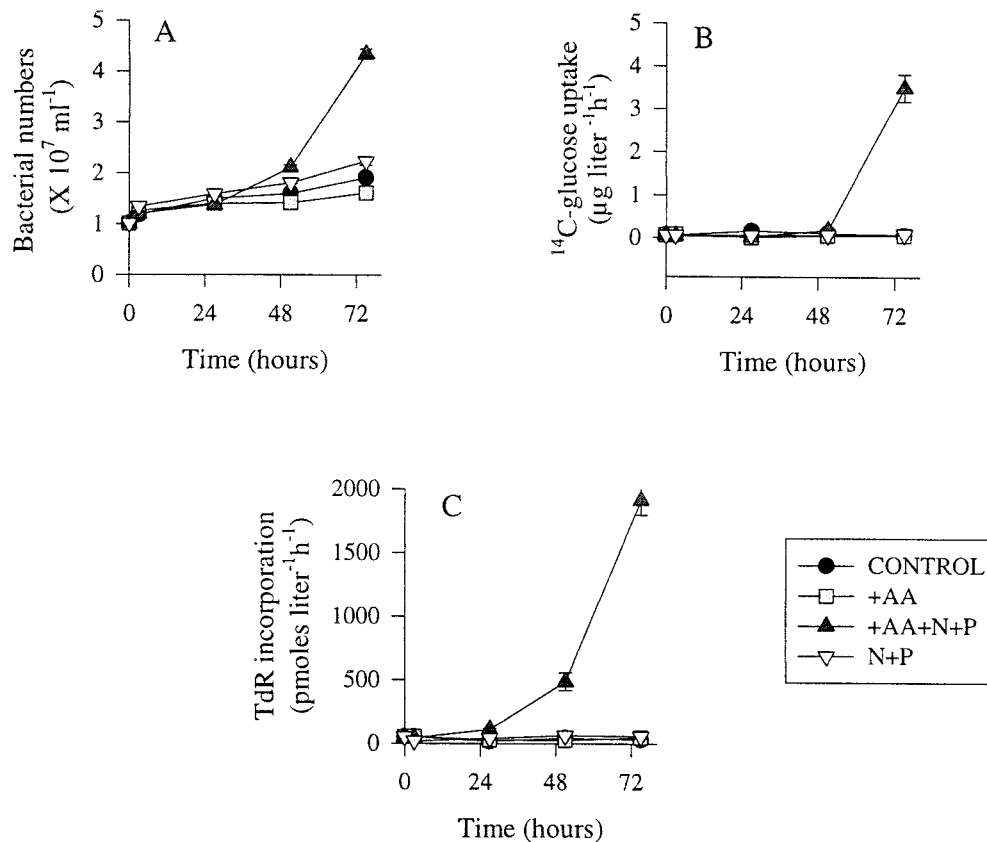


Fig. 1. Changes in bacterial numbers (A), ^{14}C -glucose uptake plus respiration rates (B), and TdR incorporation rates (C) with time in response to nutrient and organic substrate additions in the nutrient enrichment experiment, Redberry Lake, June 1993. Data are mean of three replicates ± 1 SE.

measured using a scanning spectroradiometer (Arts et al. 2000). Diffuse attenuation coefficients ($K_{\text{dUV-A}}$, $K_{\text{dUV-B}}$, K_{dPAR}) and 1% depth penetrations were calculated according to Arts et al. (2000).

Results

DOM availability experiments—In the Redberry Lake experiment there was no significant difference ($P < 0.05$) when DOC concentrations at experiment start (for nutrient and control treatments) were compared to end values. Bacterial numbers decreased in the control treatment while increasing in the nutrient treatments (Table 1) ($P < 0.05$).

In 1997, spring DOC concentrations in the three inflows were 12.8 mg L^{-1} (Oscar Creek), 13.0 mg L^{-1} (Marshy Creek) and 8.4 mg L^{-1} (Trout Pond Creek), while in July 1997 the concentrations were 17.1 , 9.4 , and 17.7 mg L^{-1} , respectively. DOC concentrations in the biologically available carbon experiments decreased over time while bacterial numbers increased ($P < 0.05$), for all inflow waters and treatments (Table 1). The decrease in DOC was greater in N+P treatments than in nonnutrient treatments for all inflows. The percent biologically available carbon was 9 (13), 12 (15), and 14 (20)% for Marshy, Oscar, and Trout Pond Creeks, respectively (numbers in parentheses represent N+P treatments) (Table 1). Carbon availability, expressed as μg

of bacterial carbon produced per mg initial ambient DOC was 0.96 for the lake water when N+P were added. In Marshy, Oscar, and Trout Pond Creeks carbon availability was 9.3 (8.7), 3.8 (5.2), and 4.4 (8.4), respectively (numbers in parentheses represent N+P treatments) (Table 1).

Nutrient enrichment experiments—For Redberry Lake samples, the combined N+P+AA additions had a clear effect on all parameters measured (Fig. 1). On the final day of the experiment, bacterial numbers, ^{14}C -glucose uptake, and TdR incorporation rates in this treatment were on average 2, 50, and 43 times higher, respectively, than in the other three treatments (Fig. 1A–C), differences which were statistically significant ($P < 0.001$). The two way ANOVA showed a significant interactive effect of nutrients with organic substrate additions. In all treatments, bacterial numbers increased significantly ($P < 0.001$), indicative that bacterivores had been reduced or excluded by the filtration treatment.

Photolysis experiments—Maximum instantaneous irradiances for one day (15 June) of the photolysis experiment were $4.4 \times 10^{-2} \text{ W cm}^{-2}$ (PAR 400–700 nm), $5.1 \times 10^{-3} \text{ W cm}^{-2}$ (UV-A 320–400 nm) and $2.0 \times 10^{-4} \text{ W cm}^{-2}$ (UV-B 280–320). Representative maximum mean instantaneous summer irradiances (June, July, August 1996; Waiser un-

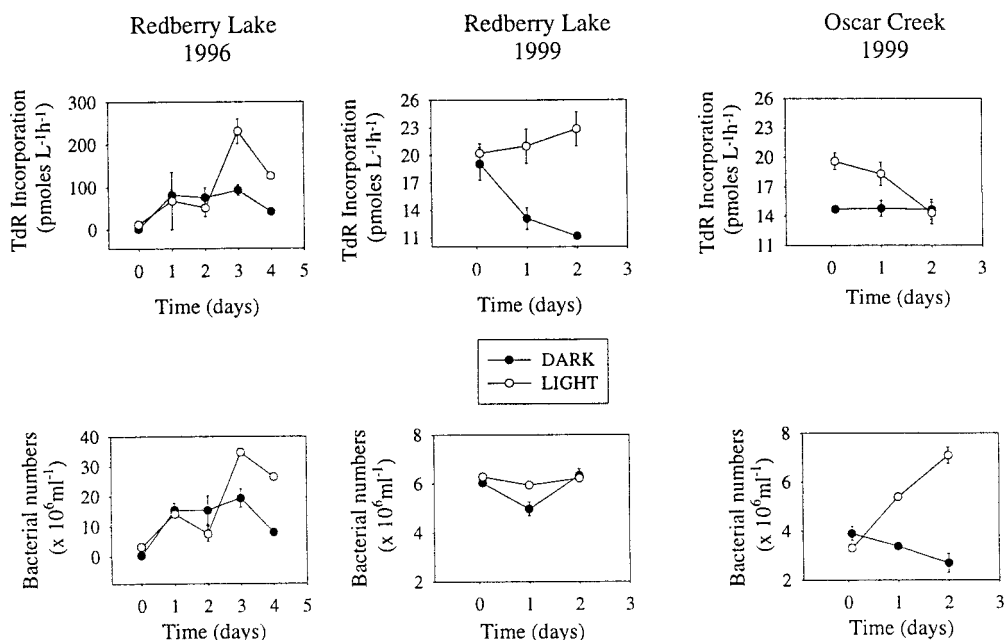


Fig. 2. Changes in TdR incorporation rates and bacterial numbers with time for Redberry Lake and Oscar Creek water with (PAR+UV-A+UV-B) and without (dark control) exposure to light.

publ. data) were $3.79 \pm 0.9 \times 10^{-2} \text{ W cm}^{-2}$ (PAR), $3.88 \times 10^{-3} \text{ W cm}^{-2}$ (UV-A), and $1.65 \times 10^{-4} \text{ W cm}^{-2}$ (UV-B). All data originated from scans taken on the rooftop of NHRC.

For the exposure phase of the photolysis experiment, the $0.1 \mu\text{m}$ tangential filtration unit removed approximately 98% of the bacteria from both Oscar and Redberry Lake water. In addition there was no significant difference ($P < 0.05$) in bacterial numbers in the incubation trays when numbers at the beginning were compared to those at the end of the five-day exposure (data not shown).

In Redberry Lake during the bacterial growth phase of the 1999 photolysis experiment, although there was no significant increase in bacterial numbers in the light treatment compared to the control, rates of TdR incorporation were significantly higher ($P < 0.05$) (Fig. 2). For Oscar Creek, rates of TdR incorporation in the light treatment were significantly higher than the dark control ($P < 0.05$) over the first two sampling periods, but gradually declined until rates in both dark and light treatments were the same by experiment end (Fig. 2). Bacterial numbers in the light treatment, however, were significantly higher than those in the dark control by day 1, a trend which continued until end of experiment (Fig. 2). Between 1995 and 1998, we conducted 20 photolysis experiments, similar to the one described here, on Redberry lake water at different times of the year. In all experiments we have seen evidence of photolytic breakdown of DOM and subsequent use by bacteria. The results of one such experiment, conducted in July 1996 have been included in Fig. 2 for comparative purposes.

DOM characterization—Approximately 69% of the DOM in Redberry Lake and 68% of Oscar Creek DOM was retained as the hydrophobic fraction (HPOA+HPON (= hydrophobic organic neutrals)) on the XAD-8 resin (Table 2).

The fraction of total DOM (as determined by tangential filtration) present in the low molecular weight fraction varied with site but was generally higher in the lake than in the inflows (Table 2). In the lake, 73% of total DOM was low in molecular weight ($<1,000 \text{ D}$). This was corroborated by electrospray mass spectrometry which showed the average molecular weight of XAD-8 extracted freeze-dried HPOA to be approximately 350 Daltons (Table 2). In Marshy, Trout Pond, and Oscar creeks, the percentages of LMW DOM were 78, 61, and 55, respectively (Table 2).

DOC peak fluorescence values were 9.8 (0.27) for Redberry Lake and 150.2 (10.20) for Oscar creek. Numbers in parentheses represent peak fluorescence normalized to ambient DOC concentration. Fluorescence ratios (450 : 500 nm) were 1.43 and 1.49 for lake and creek water respectively (Table 2).

The $\delta^{13}\text{C}$ (PDB) signature for whole water DOM and HPOA from Redberry Lake were -24.35% and -25.3% respectively, while the $\delta^{13}\text{C}$ signature of HPOA from Oscar Creek was -26.9% . ^{14}C ages of isolated DOM from the lake and Oscar Creek were 91.8 pmC (approximately 700 years old) and 180 pmC (postbomb), respectively (Table 2). The ^{14}C signature from the creek is higher than the bomb peak, indicating that ^{14}C in these samples must be derived from nonatmospheric sources. Because strictly clean procedures were used during the isolation process, it was concluded that this high value likely occurred due to spallation of naturally occurring uranium compounds in the biosphere, something which has been previously seen in samples from Saskatchewan (R. Beukens, Univ. of Toronto, pers. comm.).

The C:N ratio of lake DOM was 40:1, while the C:N ratio of DOM from Oscar Creek was 10:1 (Table 2). In Redberry Lake the relative percentage of aromatics (AR) from the NMR spectra was 9% while for Oscar Creek it was

Table 2. Characterization of DOM from Redberry Lake and its three inflows.

	Redberry Lake	Oscar Creek	Marshy Creek	Trout Pond Creek
DOC (mg L ⁻¹)*	35.0	14.9	11.2	13.1
% HPOA	69.0	68.0	ND	ND
$\delta^{13}\text{C}$	-25.3	-26.9	ND	ND
¹⁴ C (pmC)	91.8	180.0	ND	ND
% < 1KD	73.0	55.0	78.0	61.0
AVGE MW (KD)	350.0	ND	ND	ND
C:N ratio	40:1	10:1	ND	ND
% Aromatic	9.0	21.0	ND	ND
% Chromophoric	31.0	39.0	ND	ND
Peak fluorescence (324 nm)	9.8	150.2	ND	ND
Fluorescence ratio (450:500)	1.4	1.5	ND	ND

* Seasonal means.

ND, Not determined.

21% (Fig. 3; Table 2). The percentage of chromophoric carbon (i.e., carbon from the AR, C-I and C-II areas of the ¹³C NMR spectra) for Redberry Lake was 31% while in Oscar Creek it was 39%.

UV light penetration—In Redberry Lake Z_{1%} (1% light penetration) for UV-B was 0.93 m and for UV-A, 2.99 m. In the creek the corresponding values were 0.12 m and 0.30 m even though the DOC concentration was >50% lower than in the lake (Fig. 4).

Discussion

DOM origin—In aquatic ecosystems, DOM can be derived from both allochthonous and autochthonous sources (McKnight and Aiken 1998). One of the most interesting,

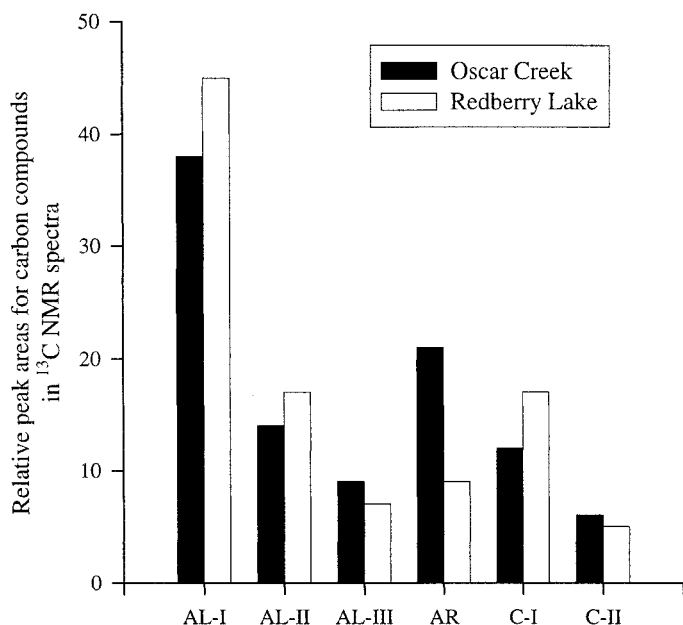


Fig. 3. A comparison of relative peak areas for carbon compounds in ¹³C NMR spectra from Oscar Creek and Redberry Lake HPOA.

and at times perplexing, questions of this study was where lake DOM originated. The source of DOM from different aquatic systems can theoretically be determined by plotting the ratio of aromatic carbon AR to aliphatic carbon AL (as determined by ¹³C-NMR) versus the atomic C:N ratio (McKnight et al. 1994). When we plotted this data for Redberry Lake it fell roughly within the area of samples from Antarctic ponds whose DOM is solely derived from phytoplankton (McKnight et al. 1994). However, when we compared the $\delta^{13}\text{C}$ signatures of DOM in the lake to that of creek DOM and soil organic matter (SOM) in the basin (Schiff et al. 1990), the $\delta^{13}\text{C}$ signatures of SOM in the basin (-25.11‰, Anderson 1995) and the inflowing HPOA from Oscar Creek (-26.9‰) matched closely with those of lake HPOA (-25.3‰). As well, the hydrophobic fraction comprised a high percentage of the DOM in both the inflow (69%) and the lake (68%). According to McKnight et al. (1994), the HPOA (fulvic acid) fraction appears to be a low percentage of the DOM in aquatic systems where DOM is derived from algal material alone. Results from their two Antarctic lakes indicated that fulvics were only 22 and 18 percent of the total DOM. Although Redberry Lake DOM was low in aromaticity, which could be indicative of an autochthonous source (McKnight et al. 1994), it had an unusually high C:N ratio which is not typically seen in autochthonously derived DOM.

As well, some of Redberry Lake DOM is old despite being continually diluted with much younger DOM from the inflow. If lake DOM was autochthonous in origin, it would have a predominantly young ¹⁴C-age provided dissolved inorganic carbon (DIC) was atmospheric in origin. This is due to the fact that all organic material produced by photosynthesis will be tagged with "bomb" carbon (Druffel et al. 1992). Although we do not have ¹⁴C-ages for either DOC excreted from phytoplankton or DIC, we do know that the $\delta^{13}\text{C}$ signature for DIC in the lake is -7. If this DIC were in equilibrium with the atmosphere it would have a $\delta^{13}\text{C}$ signature of about 0 to +3. Lake DIC, therefore, is depleted in ¹³C relative to its equilibrium with the atmosphere. As well, we calculated the mean negative log of the partial pressure of CO₂ (-log pCO₂) for the ice free season of 1998 to be 10^{-3.15} atm using the U.S. Geological Survey's NETPATH

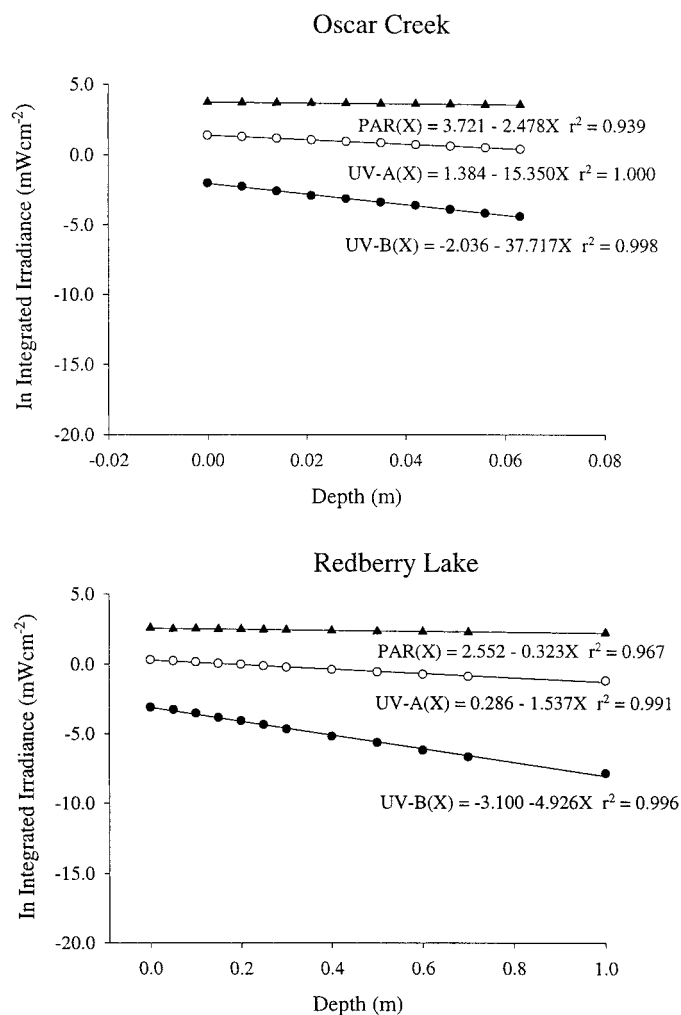


Fig. 4. Natural log of integrated irradiances at different depths for Redberry Lake (Arts et al. 2000) and Oscar Creek. Note differences in plotted depths for the two sites.

program (Plummer et al. 1994). A comparison with the atmospheric value of $10^{-3.5}$ atm reveals that some degassing is occurring in the lake. Both the $\delta^{13}\text{C}$ signature of DIC and the $-\log \text{pCO}_2$ data indicate that DIC in the lake is probably a mixture of atmospheric and nonatmospheric CO_2 (D. van Stempvoort pers. comm.). Due to the complexity of processes affecting DIC in Redberry, we are not able to estimate the significance of phytoplankton to DOC production and to the origin of lake DOM.

Perhaps the most convincing evidence regarding origin of lake DOM, comes from the fluorescence data. Allochthonous or terrestrially derived DOM has a fluorescence ratio of about 1.4 while that of microbially derived DOM has a ratio of about 2.0 (Donahue et al. 1998). The fluorescence ratio of DOM (450:500 nm) for the lake (1.42) is very close to that of creek DOM (1.49) indicating that DOM is mainly allochthonous in origin.

Despite conflicting information on the origin of lake DOM, we believe the majority of the evidence points to a terrestrial origin. Although Redberry Lake DOM plotted near the microbial end member on the C:N versus AR/AL-

I graph, its position probably results from chemical changes in DOM, rather than autochthonous origin. Donahue et al. (1998) note that photochemical oxidation of aromatic portions of DOC leaves the more aliphatic portions behind—something that we have documented for Redberry Lake DOM (Fig. 3). They have termed this type of DOC, “autochthonous-like” as it is “not a product of in-lake metabolism but rather in-lake chemical alterations”. As well, Hedges et al. (1992) have suggested that distinct compositional differences between Amazon River DOM and its sea-water counterpart could be a result of extreme chemical alteration following river discharge. The difference in carbon normalized fluorescence between creek and lake water DOM certainly supports the idea that chemical changes in lake DOM have occurred (Anesio et al. 1999).

Despite the fact that lake and creek DOM have the same source, our study clearly shows major differences between lake and creek DOM. While DOM C:N ratios of 10:1 in the creek mirror that of soil organic matter in the basin (10:1, Anderson et al. 1995), they differ greatly from the ratios of 40:1 seen in lake DOM. Moreover, lake DOM is lower in aromaticity, percentage of chromophoric moieties, fluorescence, and molecular weight than its creek counterpart. And finally, although DOC concentrations in the lake are 2.5 times greater than that in the creek, probably a result of accumulation of refractory DOC and evapoconcentration, lake water is not colored while creek water is highly so.

How then did these changes in DOM character occur? Arts et al. (2000) point out that saline lakes are often “end systems” located at the lowest hydrological level for surface waters. Moreover, they most commonly occupy hydrologically closed drainage basins. As well, Curtis and Adams (1995), in their study of Alberta saline lakes, showed that as salinity and DOC concentration increased there was a concomitant decrease in lake color and DOM molecular weight. This is in direct contrast to data from European humic lakes where the percentage of high molecular weight (HMW) DOM increases concomitantly with increasing DOC concentration (Tranvik and Jørgensen 1995). We believe that changes in DOM are strongly linked to the location of Redberry Lake within a hydrologically closed basin. DOM entering this lake is trapped; there are no outflows. The longer DOM resides within a waterbody, the greater the chance for change, be it biological, chemical and/or physical; water in lakes with retention times >1 year consequently tend to be clear (Rasmussen et al. 1989). This inverse relationship between water color and retention time has also been documented for lakes in the Swedish forest region (Meili 1992). The relatively old age of at least some of the DOM in Redberry Lake (91.8 pmC or ~ 700 years) indicates that a percentage of the DOM has been in the lake for a long time. In fact, DOM in lake water may actually be much older since the ^{14}C -age of inflowing carbon may be masked by spallation products of uranium, a major commercial resource in Saskatchewan.

We propose that over long time scales, photochemistry has played a role in changing the character of lake DOM. Such changes have been well documented in the literature (Chen et al. 1978; Miller and Moran 1997; Miller 1998). In one such study, dilute fulvic acid (FA) solutions (derived from

soil organic matter) were exposed to UV radiation (UV-R) for various time periods and at differing pH (Chen et al. 1978). At alkaline pH, the rate of photooxidation of FA was greatest and phenolic compounds disappeared from and inorganic carbonates appeared in, the irradiation products. Moreover, UV-R bleached the FA solutions until they were colorless. The authors speculated that phenolic compounds not only gave FA solutions their color but that they were destroyed by UV light. The results of our study are certainly in line with those of Chen et al. and with recent photochemical research which links DOM color to the presence of aromatic compounds, photoreactivity to chromophoric compounds and the loss of color or photofading to DOM exposure to sunlight (Vodacek et al. 1997; Clair and Sayer 1997; Miller 1998). Redberry Lake water is alkaline (pH 8.8, seasonal mean), a condition which appears to favor photochemical breakdown of DOM (Chen et al. 1978). Lake DOM is lower in molecular weight, aromatics, chromophoric moieties, fluorescence, and color than its inflowing counterpart — all of these changes are similar to changes which Chen et al. (1978) documented. Although the low aromatic content in lake DOM could be due to sorption onto oxides (McKnight, Univ. of Colorado. pers. comm.), our data are most consistent with changes in lake DOM mediated through photochemical activity.

Such photochemically induced changes in DOM have had a tremendous impact on the optical properties of Redberry Lake water and are responsible for the major differences in the capacities of lake and creek water to attenuate UV-R. In aquatic systems, both the concentration of DOC and the relative percentage of chromophoric compounds are factors influencing the attenuation of ultraviolet radiation (Scully and Lean 1994; Clair and Sayer 1997) and therefore any changes in DOM character will have an effect on water optical properties (Miller 1998). For example, Vodacek et al. (1997), in their study of seasonal variation of chromophoric dissolved organic matter (CDOM) and DOC in the Mid Atlantic Bight, documented a 70% loss in absorption and fluorescence of CDOM in surface waters during August. They attributed this dramatic change in optical properties to photobleaching. Similar changes are seen when the optical properties of DOM from Redberry Lake and Oscar Creek are compared. For example, carbon normalized peak fluorescence of lake DOM is 97% lower than that of the creek. Moreover, although DOC concentrations in Redberry Lake are greater than those in the inflow, the depth of 1% surface irradiance ($Z_{1\%}$) for UV-A and UV-B radiation is 10 and 8 times greater in Redberry Lake than in the creek. This is due to the lower percentage of chromophoric compounds in lake DOM. Changes in lake water optical properties can also be illustrated when the diffuse attenuation coefficients (K_d) for UV-A, UV-B and PAR from Oscar Creek, Redberry Lake, and two other prairie aquatic systems — Deadmoose Lake (35.7 mg L⁻¹ DOC) and Pond 4857 (37.6 mg L⁻¹ DOC) (data from Arts et al. 2000) are normalized to DOC concentrations and then compared (Fig. 5). Deadmoose Lake (saline) and Pond 4857 (fresh water) were chosen because they have DOC concentrations similar to that of Redberry Lake yet are more typically humic-stained water bodies. When the comparisons are made, the differences are dramatic, showing that on a

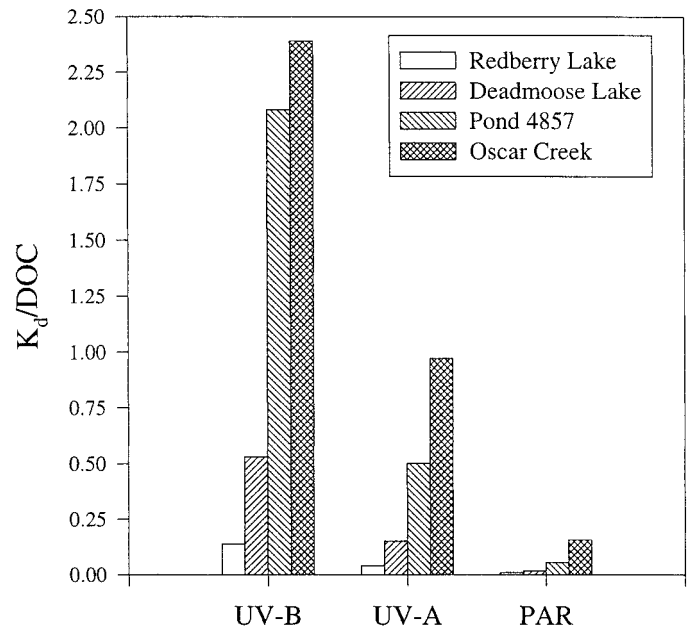


Fig. 5. A comparison of K_d values (UV-A, UV-B, and PAR) from Redberry Lake, Deadmoose Lake, Pond 4857 and Oscar Creek normalized to ambient DOC concentration.

carbon normalized basis, DOC in Redberry Lake attenuates UV-R to a far less extent than DOC in other prairie systems (Fig. 5).

Photochemical activity could have other effects on lake DOM as well. For example, complete photooxidation of some of the DOM to dissolved inorganic carbon is likely. Model results from the study of Vodacek et al. (1997) indicated that approximately 10% of the DOC in the mixed layer on the Mid Atlantic Bight was photooxidized to DIC. If such photolytic processes were at work, it might be expected that during the ice-free season, concentrations of DOC in the epilimnion of Redberry Lake would be lower than in the hypolimnion. In the winter, under ice cover, there should be no difference. Over a two-year period, epilimnetic DOC concentrations were statistically lower (6.5%) than hypolimnetic concentrations, while there were no such differences in winter (data from Robarts et al. 1999). Although there are other possible explanations for these data, they are consistent with photooxidation of a portion of DOM to DIC.

Results of our photolysis experiments also suggest that photolytic breakdown of DOM is occurring in both lake and creek water. In these experiments, although the timing and the type of the response was different, bacteria in the light treatments responded to photolytic production of low molecular weight carbon compounds, as demonstrated by either an increase in TdR incorporation or bacterial numbers, phenomena which have been noted in other studies (e.g., Moran and Zepp 1997).

The differences noted in the C:N ratios of lake and creek DOM seem to suggest that over time some process has been stripping nitrogen from lake DOM. One possibility could be that bacterial processes have been at work, breaking down the more labile and suitable (in terms of C:N ratio) carbon compounds (Kroer 1993), utilizing nitrogen in the process.

This explanation is not supported by the results from our microcosm enrichment and biologically available carbon experiments. Rather, these experiments indicate that although bacteria in the lake are physiologically capable of taking up labile organic substrates when nutrient limitation is alleviated, low molecular weight (LMW) carbon, which dominates the bulk DOC pool in the lake, appears to be largely unsuitable for microbial growth.

Instead, we believe that nitrogen loss from lake DOM is probably coupled to photolytic loss of aromatic moieties. McKnight and Aiken (1998) note that nitrogen loss from aquatic DOM appears to occur at similar rates to photolytic oxidation of aromatic moieties. We have shown that lake DOM is 57% lower in aromatics than its creek counterpart. Moreover, direct photochemical release of ammonium from DOM has been demonstrated by Bushaw et al. (1996) after wetland water was exposed to full sunlight. In 1996 we carried out some limited nutrient analysis of Redberry Lake water before and after exposure to full sunlight during photolysis experiments and found preliminary evidence for photolytic generation of ammonium. Also interesting is the fact that ammonium concentrations in the lake are approximately 15 times higher than those of nitrate/nitrite (Roberts et al. 1992). Although the ammonium release data must be interpreted with caution (the analyses are from one experiment only and not replicated), the observations raise the intriguing possibility of photolytic release of ammonium from aromatic constituents of lake DOM which could explain the high C:N ratios observed. More in-depth photochemical studies, which could elucidate actual photochemical rates of degradation and the type and apparent quantum yields of photooxidation products, are needed.

Effects of DOM character on microbial carbon cycling—According to Cole (1999), one of the largest fluxes of carbon in almost any ecosystem is that from the organic matter pool into microorganisms. DOM in Redberry Lake, therefore, represents a potentially important carbon and energy source for aquatic bacteria. Our study results indicate that a portion of lake DOM is photoreactive and that LMW carbon compounds thus generated can be utilized by bacteria for growth. How important this is in terms of overall carbon cycling is not known at this time. We do know, however, that the majority of DOM in Redberry Lake is unavailable for bacterial growth and therefore photoproduction of labile carbon may be important in terms of overall productivity (Miller and Moran 1997).

In aquatic systems, bacterial growth rates may be limited not only by the quantity, but also the quality of DOM (Kirchman 1990). Although photochemistry has produced LMW compounds suitable for bacterial growth in Redberry Lake, we believe that it has also changed the composition of lake DOM thereby diminishing its quality as a bacterial substrate. Phototransformation of DOM to biorefractory substrates has been shown in other studies (Amon and Benner 1996; Anesio et al. 1999). The short-term enrichment experiment showed bacterial growth on DOM in the lake was low with or without addition of N+P. This experiment also demonstrated that lake bacteria were physiologically capable of growth when provided with labile nitrogen, phosphorus, and

organic substrate ruling out the possibility that high salinity and alkalinity in the lake were somehow responsible for low bacterial growth. When carbon availability (measured as μg bacterial carbon produced per mg of ambient DOC) for Redberry Lake and Oscar Creek were compared, inflowing higher molecular weight material was 8 times more available to bacteria than lake DOM. The experimental results suggest that although Redberry Lake bacteria are physiologically capable of growth, LMW DOM in the lake is less suitable as a bacterial substrate than its creek counterpart. Until recently, it was generally accepted that LMW carbon substrates were preferential substrates for aquatic bacterial growth (Leff and Meyer 1991). However, in marine environments bacteria preferentially degrade HMW organic matter, a process which over time results in a large pool of relatively nonreactive, LMW carbon (Amon and Benner 1994). A similar process appears to be operational in Redberry Lake, although mediated mainly through photochemistry, not bacterial degradation. Regardless of the process, the end result is that the majority of DOM in the lake is low in molecular weight and has been processed to the point where it is biologically nonreactive. The old age of a portion of the DOM in Redberry Lake provides support for the existence of an “old background of organic matter which is resistant to in situ microbial oxidation” (c.f. Druffel et al. 1992) and which cycles slowly over long time scales.

Relatively little is known, however, about hydrophilic organic acids which comprise 31% of the DOM in this lake. These hydrophilic acids contain proteins, carbohydrates as well as free sugars, and amino acids and it may be that this DOM is a younger, more reactive component (biologically and photochemically) which cycles on faster time scales than the older material (Schiff et al. 1990; Druffel et al. 1992). Further research into the ^{14}C ages of the various carbon compartments is needed to establish the role that this DOM fraction plays in overall carbon cycling in this lake.

In summation, this study demonstrates the influence of both hydrologic conditions and photolysis on the chemical structure and biological lability of DOM. Despite their common origin, there are significant differences between DOM from Redberry Lake and Oscar Creek. DOM photoreactions have had a major chemical and biological impact on Redberry Lake. Over short time scales, photochemical reactions have produced LMW carbon compounds suitable for bacterial uptake. Over longer time scales, these reactions have degraded DOM to the point where the majority is compositionally unsuitable as a bacterial substrate. Not only do our data show the impact of photochemical processing on the chemical characteristics of DOC trapped in endorheic saline lakes, but also the impact this has on water optical properties and the underwater UV climate in these systems. Williamson et al. (1996) in their study of UV radiation in North American lakes warned that attenuation depth estimates, based on DOC concentrations, were only first-order estimates and did not take into consideration differences in the optical characteristics of different types of DOC. Our study provides one mechanism to account for the remarkably different optical properties of DOC in lakes of the northern plains region as recently documented by Arts et al. (2000).

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