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Low-Temperature Microbial Activity in River Systems

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PURPOSE: This technical note examines dissolved oxygen (DO) levels and changes in river microbiology during winter, low temperatures, and periods of ice cover with the objective of providing guidance for winter water quality modeling. The results of this study provide information on cold-water aquatic microbial activity and the related coefficients necessary to model such activity. The product of this research can be applied immediately to improve winter modeling using existing water quality models such as the Corps' CE-QUAL-W2.

BACKGROUND: Adequate levels of DO are critical to sustain aquatic life and maintain acceptable water quality. The most critical period for dissolved oxygen deficits is often assumed to be summer, because low flows often coincide with high water temperatures. However, previous research has documented the occurrence of wintertime oxygen deficits in ice-covered rivers. DO deficits have been known to coincide with ice cover formation (Schreier et al. 1980; Whitfield and McNaughton 1986) and DO deficits can increase until ice cover breakup (Shallock and Lotspeich 1974). Rapid decreases in winter DO have been observed at river-reservoir confluences (James et al. 1992, McBean et al. 1979), with large oxygen deficits reported in deep stratified reservoirs (Gamble 1971).

Despite these observations, dissolved oxygen processes in ice-covered rivers have received little attention. Given that the solubility of oxygen in water is higher at lower water temperatures experienced during winter, the same magnitude of oxygen depletion due to biochemical oxidation has less effect during the winter than during the summer. In addition, the microbiological processes involved in the consumption of oxygen through the decay of organic matter are thought to be insignificant during winter since they are temperature-dependent, with slower rates at lower temperatures. Reaeration during winter is often assumed to be at a minimum because ice covers might provide a barrier to reaeration. They are also thought to prevent or minimize photosynthesis through the absorption or reflection of solar radiation in the form of visible light.

However, low-temperature microbial activity that occurs for extended periods in ice-covered rivers and reservoirs can have a significant impact on water quality due to decreases in DO levels (Reitner et al. 1997, James et al. 1992, McBean et al. 1979). This effect can be magnified in rivers that experience low flow during winter. Changes in a river's microbial diversity and community structure that may occur during fall, winter, and spring temperature cycles have not been well-characterized. Seasonal microbial community structure changes might influence the dominant metabolic processes occurring in a river. Degraded water quality manifested as reduced DO can result from shock loadings that occur during periods when the stability of a river's microbial community is low because of the lack of diversity. Shock loadings include rainfall or snowmelt runoff containing high biochemical oxygen demand (BOD) highway and airfield deicers, as well as unusually large outflows from combined sewer systems. Understanding the relationships among seasonal river changes and river microbial community structure will lead to improved predictive capabilities for winter water quality.

Quantitative wintertime DO modeling to examine winter effects is difficult because conventional water quality models (e.g. U.S. Environmental Protection Agency's (EPA's) QUAL2E (EPA 1995) and the Corps of Engineers' CE-QUAL-W2 (Cole and Buchak 1995)) are extremely complex. They require calibration data that, while sometimes difficult to obtain in open-water conditions, are generally unavailable for ice-covered rivers. Water quality models also provide uncertain results during the winter because they neglect the effects of reaeration and deoxygenation at the low temperatures occurring during winter. The inclusion of winter dissolved oxygen processes in simpler models will allow greater flexibility in analyzing and predicting riverine water quality.

Riverine microbial communities in winter may be different than those in summer, and these differences may have a greater effect on water quality than would be predicted by temperature differences alone. Colder water temperatures would be expected to reduce microbial activity in winter. An approximate estimate of activity of a "pure" biologically mediated process at lower temperature could be obtained from the Arrhenius equation:

$$K = Ae^{-Ea/RT} \tag{1}$$

where A is a constant, E_a is the activation energy, R is the universal gas constant, and T is the temperature. For many biochemical reactions, this relation yields a doubling or halving of rates as temperatures increase or decrease, respectively, by 10 °C. In practice, microbial activity in rivers and subsequent effects on water quality are assumed to be insignificant at temperatures below about 5 °C.

Other factors, such as river flow rates, nutrient loads, DO levels, ice cover, increased surface area due to ice cover and frazil ice, and temperature cycles are influenced by winter conditions. Less is known about interactions among these factors and microbial influences on water quality. These winter phenomena can impact microbial communities. Frazil ice, for example, may increase surface area sufficiently to have a significant effect on biofilm development and this may alter water quality in winter conditions. Winter conditions may result in different microbial communities, which in turn may result in changes in the dominant metabolic processes in a river. Such changes may affect winter river water quality in addition to temperature-driven rate changes.

In previous studies using soil samples, we have shown that the effects of temperature cycling on microbial activity are not intuitive (Reynolds et al. 1998). Using a diurnal cycle with an amplitude of 20 °C, we observed no significant difference in cumulative CO_2 evolution between constant and cyclic diurnal treatments when soil temperatures were always above freezing. For the soil and the conditions we imposed, using the mean daily soil temperature to predict CO_2 evolution appears to be appropriate when temperatures are above freezing.

However, when the minimum diurnal temperature reached below freezing, even though the mean temperature was above freezing and the amplitude in the diurnal cycling again was 20 °C, CO_2 evolution increased in the cyclic diurnal treatment relative to the equivalent constant temperature. In such cases, CO_2 evolution predictions based on daily mean temperatures may underestimate CO_2 evolution.

Synthesizing the above observations resulted in the following hypotheses: 1) The effects of cyclic temperatures on biologically governed processes in streams and rivers may be greater than are currently recognized, 2) Omitting low-temperature effects on microbial process rates may result in inaccurate predictions of water quality, and 3) Temperature-mediated changes may influence not only rates, but may also alter the dominant microbial community, thereby causing changes in the primary metabolic pathways.

Unfortunately, data that can be used in the development and testing of hypotheses related to winter DO are sparse. The published data that do exist for a few northern rivers in North America (including the Athabasca River in Canada and the St. John and Yukon Rivers in the United States and Canada) are characterized by wide spatial and temporal variations in measurement. Recently, one-day winter (January and March) DO and photosynthetically active radiation (PAR) data have been reported by Guasch et al. (1998) for two rivers in Spain; however, no ice cover was present. White and Melloh (1999) report high-temporal resolution data for a small river in Vermont, but the relatively pristine conditions there do not yield data useful in modeling more nutrient-rich river systems.

This study provided high temporal-resolution water quality data for an intermittently ice-covered, relatively nutrient-rich river in Vermont. Field data and laboratory studies were performed to evaluate reaeration and deoxygenation coefficients, and microbial diversity was examined.

METHODS:

Field: Hypotheses were tested using a combination of field and laboratory experiments. Field data were collected from late fall 1999 through spring 2000 on the LaPlatte River at Hinesburg, VT. The LaPlatte River watershed is 53 square miles, of which 47.5 percent is agricultural, 40 percent forested, and 8 percent urban. Elevations range from 1600 ft mean sea level (MSL) near the headwaters in Hinesburg to about 310 ft MSL near the Hinesburg Wastewater Treatment Plant, to about 100 ft MSL at the confluence with Shelburne Bay in Lake Champlain. Water quality data were collected immediately downstream from the outfall of the Hinesburg Wastewater Treatment Plant, which consists of aerated lagoons in series followed by a chlorine contact chamber. The watershed at the sampling point is approximately 18 square miles, primarily forested with limited agricultural, residential, and commercial development. At this location, the river is gently sloped and meandering, with silty clay substrate. The river is surrounded by agricultural land (corn and pasture) near the sampling site. A cheese plant is located approximately 0.6 mile upstream and effluent from the cheese plant enters the Hinesburg Wastewater Treatment Plant after treatment in oxidation ditches followed by clarification.

A YSI 600X probe was initially placed at the center line of the river. Total river depth was approximately 1 m. Floats were added to the sensor to keep it from dropping to the bed during low flows and the resulting initial probe depth was approximately 0.3 m. The depth of the probe varied with flow during the course of the sampling period. The probe included sensors measuring DO (mg/l and percent saturation), water temperature (°C), conductivity (mS/cm), and pH. The probe was calibrated at the Cold Regions Research and Engineering Laboratory (CRREL) prior to installation. DO at the time of calibration was saturated at the local temperature and pressure. A three-point pH calibration was made using buffers of 4.01, 7.0, and 10.00. Specific conductivity was calibrated using a 1.0-mS/cm solution. Post-calibration was performed in the field after breakup. A factory-

calibrated LICOR spherical quantum sensor (LI-193SA) measuring PAR ($\mu mol/m^2$ s) was placed at the same location.

Both sensors were connected via cable to a Campbell CR10X data acquisition system located on the riverbank (Figure 1). The data were collected at 10-min time intervals for storage in the data acquisition system. Stored data were accessed and downloaded by computer via a modem and cellular phone connected to the data acquisition system (Figure 2) at intervals ranging from daily to every seven days. A solar cell and battery provided power for the system. Air temperature and battery voltage were also monitored. Ice conditions (including depth of snow on ice, ice morphology, and bottom surface structure) were monitored periodically. The data were used to explore the effects of coefficients of reaeration on dissolved oxygen during the winter period.

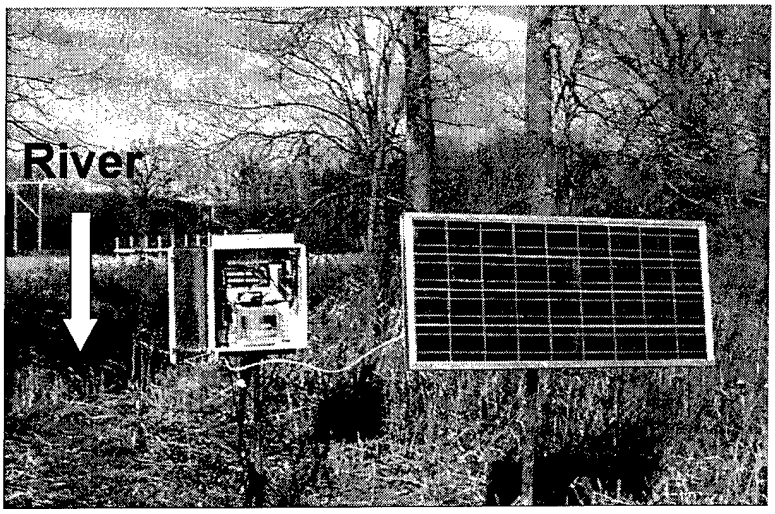

Figure 1. Data acquisition system and solar cell on bank of LaPlatte River, Hinesburg, VT

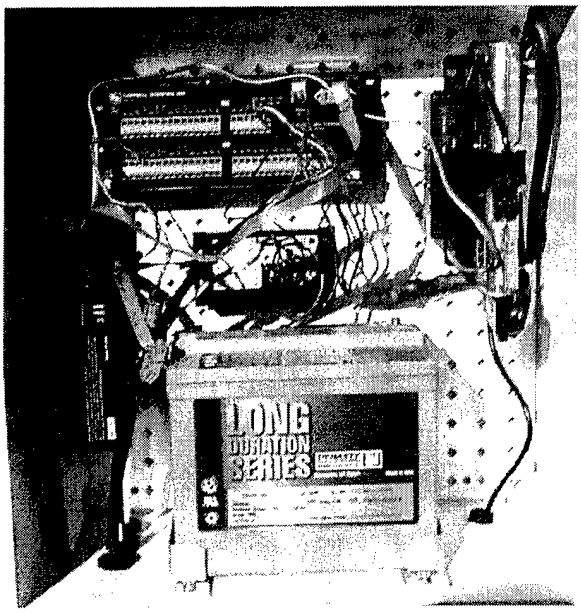

Figure 2. Data acquisition system showing (clockwise from top) Campbell CR10X, cell phone, battery, and modem, with voltage regulator in the center

At intervals, water samples were obtained, stored in sterile gallon bottles, and transported to CRREL for further testing. Microbial samples were also collected by suspending a glass-bead packed tube in the water during open-water periods (Figures 3 and 4). The purpose of this sampling approach was to obtain a biofilm on the glass beads for characterization, in addition to the open-water samples. The microbial samplers were autoclaved prior to sampling. Microbial samples were obtained by aseptically rinsing microorganisms from the beaded columns with a known volume of sterile water into a sterile vessel.

Laboratory: The laboratory portion of the study used CRREL's temperature-controlled respirometer (Figure 5). Respiration measurements using evolved carbon dioxide (CO_2) are an estimate of general, non-photosynthetic, microbial activity. The respirometer was modified to allow for simultaneous monitoring of samples incubating at different temperature regimes. Each temperature regime can be cycled in a defined amplitude and

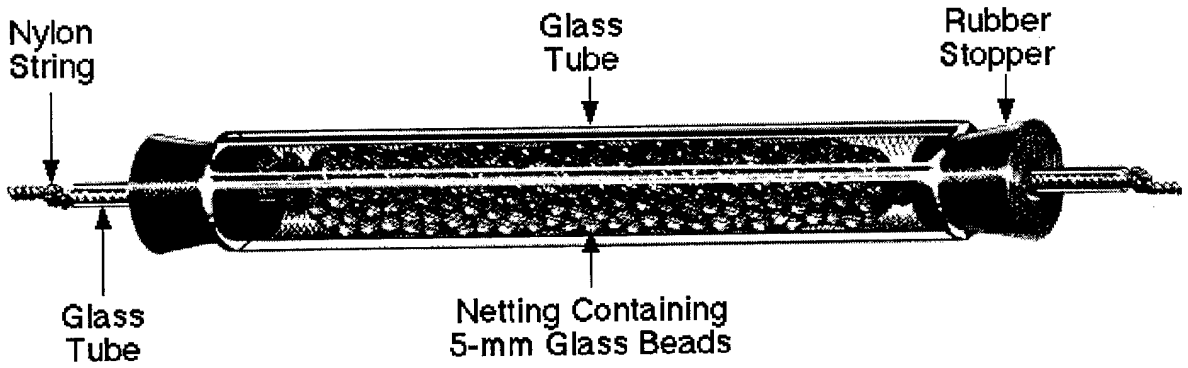

Figure 3. Flow-through sampler designed to collect microbial samples. Rubber stoppers are pulled back when submersed to allow flow through and permit deposition on the surface provided by the glass beads

Figure 4. Flow-through microbial sampler about to be suspended in open water upstream from the water quality sensors. Note sheet ice growth in river; frazil ice was also present in deposits beneath the sheet ice

frequency schedule or held constant. Samples are incubated under low-light conditions. The respirometer allows monitoring activity in near real time by taking CO_2 headspace samples approximately every 4 hr. Results from a range of constant temperature incubations may be useful for predicting fall, winter, and spring processes for a river. More commonly, the cumulative CO_2 evolution curves and existing models could be used to describe microbial activity. That is, respiration

at a single temperature would be used in conjunction with Arrhenius relationships (e.g., Equation 1) to predict respiration at other temperatures.

Respiration tests were conducted to estimate if river microbial community structure varied during the winter. The tests used water samples collected for intermittent and permanent ice cover conditions (6 January and 15 January 2000) from the LaPlatte River. CO_2 evolution at 1°C and 25°C was continuously monitored. Appropriate sterile controls were included so fluctuations caused by temperature-dependent CO_2 solubility in water could be subtracted.

In addition to general heterotrophic activity estimated from the CO_2 evolution measurements that are described above, microbial diversity was characterized and compared by a modified fatty acid methyl ester (FAME) procedure using whole cells. This approach indicates bacterial community structure changes that could be used in estimating changes in the bacterial diversity of the river system.

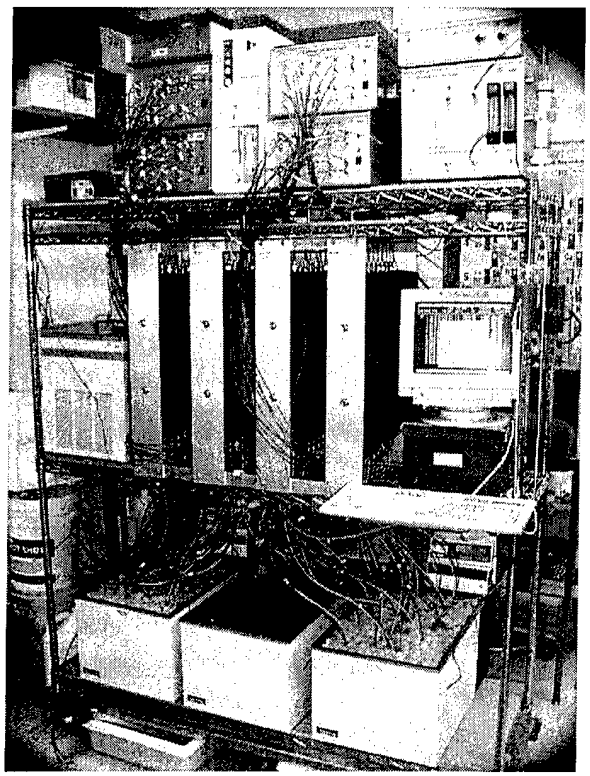

Figure 5. CRREL's temperature-controlled respirometer

The community structure in the LaPlatte River was compared on three sampling dates (February 25 and 29 and April 24) by a fatty-acid-based technique that uses culturable microorganisms recovered from the glass-bead sampling tubes. These sample times represented three conditions: when ice cover is well-established (25 February), immediately following ice breakup (February 29), and after ice out (24 April). In brief, sampling tubes (Figure 3) were brought to the laboratory and stored at 4°C. For community structure comparisons, the rubber stoppers were removed from both ends of the glass tube and the tube ends were flame-sterilized. Using aseptic techniques, 5 ml of sterile, de-ionized water was slowly introduced into the upper end of the tube. This served as a washing solution to remove the microorganisms from the beads. The washing solution was uniformly spread onto the surface of the beads in the end of the packed sample tube to limit channeling through the glass beads. Washing solution from the samplers was collected into a 40-ml, sterile vial. After the water was collected, the 40-ml collection vial was sealed.

For plating, the 40-ml vial was shaken to uniformly mix the sample and 1.0 ml of the sample was removed for making serial dilutions. For each serial dilution, 0.1-ml aliquots were spread-plated, in triplicate, onto prepared, 0.1X tryptic soy agar (TSA) plates and incubated at 25°C.

Plates were counted to estimate culturable microorganisms. For FAME characterization, the procedure used by Microbial Identification Instrument (Newark, DE) (MIDI) (Sasser and Wichman 1991). The standard procedure is a five-step process:

- Cell harvesting: Cells are harvested to yield sufficient fatty acids to be readily measurable.
- Saponification: Cells are subjected to a strong methanolic base and heat. This kills and lyses the cells, cleaving fatty acids from the cell. The free fatty acids are converted to their sodium salts.
- Methylation: Fatty acid salts are esterified by adding a methyl group to increase volatility for chromatographic analysis.
- Extraction and base wash: Free fatty acids and cellular debris are removed from the sample.
- Analysis: The fatty acid sample is analyzed by gas chromatography and the resulting fatty acid profile is compared to a library.

For the purposes of this study, the entire lawn of microorganisms was treated as a sample, rather than individual isolates. Principal component analysis (PCA) techniques were then used to compare samples.

At the initial sample collection event, the effects of the duration that the packed-bead samplers remained in the river was evaluated. The purpose of this was to estimate the time needed to develop a biofilm on the beads. A control (no river contact), and packed-bead samplers that had been submerged for 0.5 and 3.0 h were evaluated.

PCA was used to compare fatty acid profiles among sample times. PCA is a statistical technique that is used to extract the main relations in data of high dimensionality, as is found in comparing fatty acid profiles obtained by gas chromatography (GC) analysis.

RESULTS AND DISCUSSION: Water quality data were collected continuously at the Hinesburg site between 22 December 1999 and 31 March 2000. Intermittent ice covers occurred between 24 and 26 December, and on 28 December, 29 December, 31 December, and 6 January. A permanent seasonal ice cover began on about 12 January 2000. By 15 January, the ice cover was 10 cm in thickness and substantial amounts of frazil ice were deposited beneath the solid ice. Algae were visible both within the frazil ice and in accumulations at the surface in open-water areas (Figure 6). This ice cover remained in place until between 27 and 28 February 2000 when the ice cover broke up following a snowmelt and rainfall event. Open water was observed on 29 February.

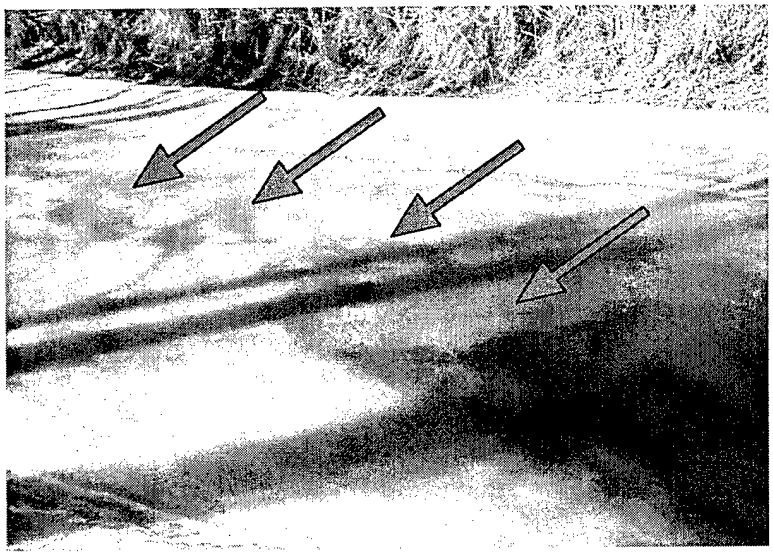

Figure 6. Algae visible at the surface at the ice/open water interface, and beneath the ice cover downstream (see arrows above). Frazil ice was packed to the bed along the banks

Water quality data. The water quality data for ice-covered and non-ice-covered periods are presented

in Figure 7. Statistical analyses were performed using SAS® statistical analysis software (SAS® Institute 1996). The sample populations for all of the measured variables are statistically significantly different ($p \leq 0.05$) between the ice-covered and open-water periods as determined using the Wilcoxon rank sums test. Water temperature, DO, pH, and conductivity conditions observed during the ice-covered period are relatively stable compared to open-water conditions and are similar to those reported by White and Melloh (1999). The data demonstrate a stabilizing effect of ice cover on water quality parameters that may provide some biological benefits to lotic communities. This idea was suggested by Hynes (1970); i.e., ice covers provide some biological benefits to lotic communities through stabilization. Hynes also noted that scour induced during ice cover breakup

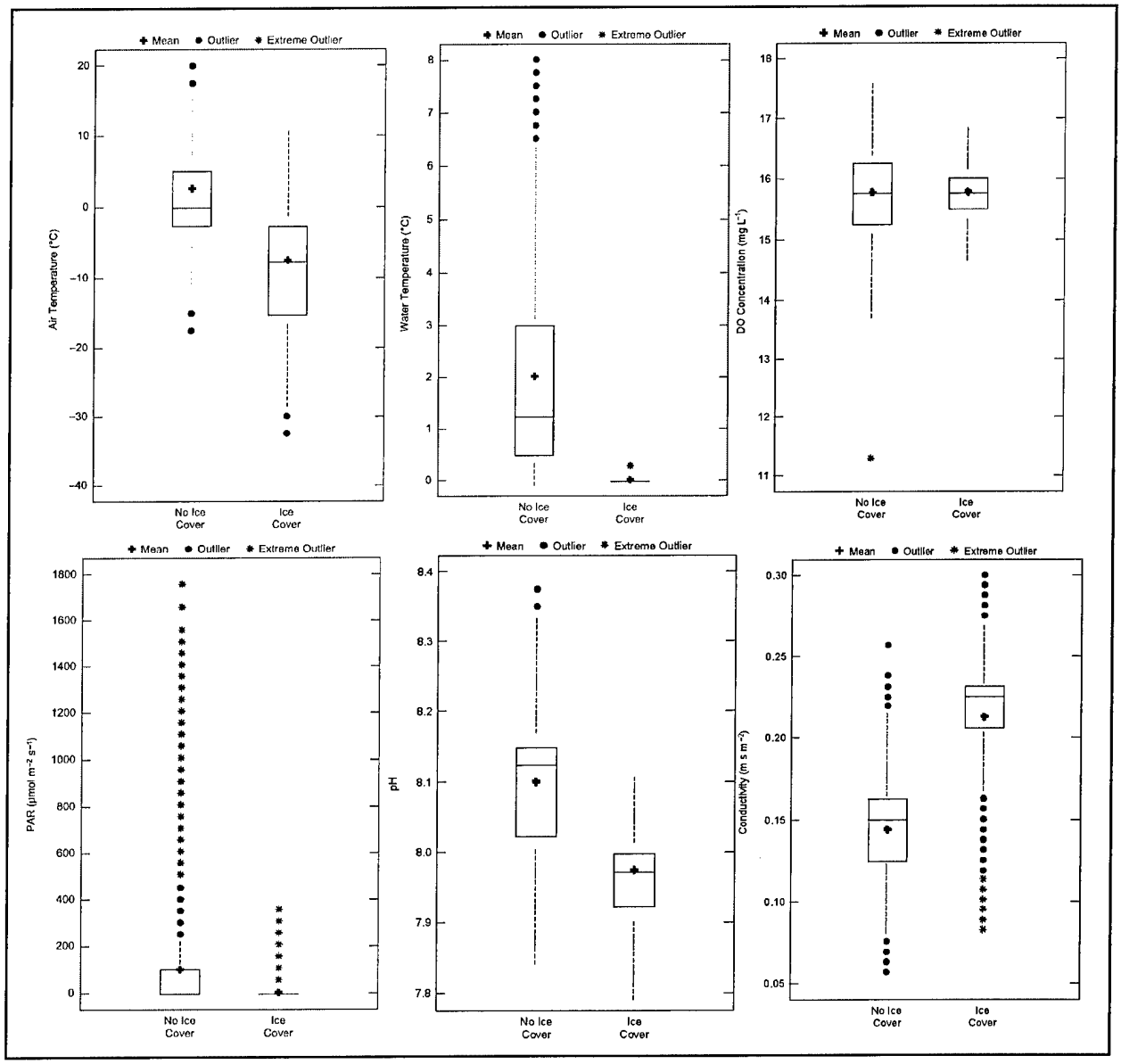

Figure 7. Data for open-water versus ice-covered periods, 22 December 1999 through 31 March 2000. Mean value is indicated by + in box, which depicts 25th percentile (lower line), 75th percentile (upper line), and median value (center line)

may hold some hazards to riverine communities; this study shows that high variability in temperature, DO, pH, and conductivity is also associated with breakup.

Descriptive statistics for the water quality variables are provided in Table 1. Correlation tests were also performed and during the open-water periods, water temperature was moderately correlated with DO percent saturation (r^2= 0.53), pH (r^2= 0.69), and air temperature (r^2= 0.66). Percent saturation DO was moderately correlated with pH (r^2= 0.77). Conductivity and pH were also correlated with depth (r^2= -0.57 and -0.70, respectively).

Table 1
Descriptive Statistics for Water Quality Data

Variable	N	Mean	Std Dev	Minimum	Maximum
Open-Water Period					
Water Temperature (°C)	6747	1.93	1.83	-0.02	8.03
Conductivity (mS)	6747	0.15	0.03	0.06	0.26
DO (Percent saturation)	6747	113.2	5.96	89.60	127.90
DO (mg/L)	6747	15.68	0.78	11.16	17.57
Depth (m)	6747	0.23	0.15	-0.04	0.93
pH	6747	8.10	0.09	7.84	8.38
PAR (µmol/m^2)	6747	87.91	168.05	0.60	1731.00
Air Temperature (°C)	6747	1.67	6.23	-18.54	19.37
Ice-Covered Period					
Water Temperature (°C)	7733	0.01	0.03	-0.06	0.20
Conductivity (mS)	7734	0.21	0.04	0.08	0.30
DO (Percent saturation)	7734	107.54	2.73	100.60	114.60
DO (mg/L)	7734	15.70	0.40	14.66	16.76
Depth (m)	7734	0.39	0.19	0.21	1.39
pH	7734	7.97	0.06	7.81	8.10
PAR (µmol/m^2)	7734	10.36	21.97	-2.39	348.10
Air Temperature (°C)	7734	-8.68	8.30	-32.54	9.66

The PAR measurements during open-water periods indicate that adequate incident sunlight is reaching the sensor level (approximately 1 ft deep) to allow for photosynthetic activity. Open-water mean integrated irradiance was 7.6 mol/m^2 day, with a daytime average integrated irradiance of 16 mol/m^2 day. The maximum observed PAR was 1731 µmol/m^2 s on 18 March. These levels are comparable to open-water wintertime values reported by Guasch et al. (1998) as well as to summertime measurements for shaded rivers.

During the ice-covered period, the mean integrated irradiance was 0.9 mol/m^2 day, with a maximum observed PAR of 348 µmol/m^2 s on 24 December. Following the formation of the permanent seasonal ice cover on 12 January, the mean integrated irradiance was 0.7 mol/m^2 day. The daytime average integrated irradiance was 1.4 mol/m^2 day, with a mean observed PAR of 16.2 µmol/m^2 s. The maximum observed PAR was 269 µmol/m^2 s on 13 January, just after ice cover formation. These values are somewhat lower than those reported by White and Melloh (1999): mean daily integrated irradiance of 3.3 mol/m^2 day and mean and maximum PAR of 488 µmol/m^2 s, perhaps because of the presence of frazil ice deposited beneath the ice cover.

During the ice-covered period, poor correlations were found among the variables water temperature, PAR, DO, pH, and conductivity, except in three cases: depth was found to be moderately correlated with DO concentration, (r^2= 0.50), DO percent saturation (r^2= 0.50), and pH (r^2= -0.56).

In comparison, Vincent et al. (1998) reported PAR values of about 80 µmol/m^2 s at 10 m (32.8 ft) depth in Lake Vanda and about 8 µmol/m^2 s at 5 m (16.4 ft) depth in Lake Bonney, both freshwater Antarctic Lakes exhibiting primary productivity. Thus, despite the relatively low PAR levels observed in the LaPlatte River during the ice-covered period, photosynthetic activity could be expected to continue. In fact, Marchand (1996) indicates that some aquatic plants appear to adapt to photosynthesis under low light levels, and Guasch et al. (1998) suggest the same for other biota.

The range of water temperature variation was small during the ice-covered period, less than 0.2 °C per day. Supercooling (water temperatures slightly less than 0 °C) was noted during the December and January open-water periods, consistent with observations of frazil ice beneath the ice cover near the sampling site. Supercooling was also noted during the early part of the ice-covered periods, indicating that turbulent open-water areas existed upstream at the time.

The observed DO levels were supersaturated during the ice-covered period, averaging 15.7 mg/L and 107.5 percent of saturation. These values compare favorably to the data reported by White and Melloh (1999), which averaged 16.4 mg/L and 113.7 percent of saturation during the late winter ice-covered period. The generally high DO levels may reflect highly efficient reaeration at low temperatures, photosynthetic input, or both. Diurnal cycling of DO was observed throughout the ice-covered period.

The low-amplitude DO fluctuations observed are comparable to those reported by Simonsen and Harremoes (1978) and White and Melloh (1999). Fluctuations in water temperature do not fully explain the observed cycling, indicating a biological component. Typical diurnal DO cycles during the ice-covered period resemble that shown in Figure 8, for 20 January 2000. DO levels are lowest during the nighttime hours, begin to rise through the morning hours, and peak sometime in early to mid-afternoon. As the ice-covered period progresses, the DO cycle becomes less well-defined (Figure 9), but the trend remains the same. The December open-water curves resemble the typical curve shown in Figure 8, as do those for April and May.

However, DO cycling during the period following breakup (after 28 February) does not follow this trend. Instead, DO reaches its lowest point at around sunset and rises to peak in the morning (Figure 10). DO concentration and water temperature are also much better correlated during this period (r^2= -0.82) than during the ice-covered period (r^2= -0.08), since the barrier to heat transfer provided by the ice is absent. Following ice cover breakup, DO peaks earlier in the day than during the ice-covered period. This is an indication that ice cover breakup induces changes in the system that drastically reduce microbial impacts. Such changes could include the loss of microbial communities living within the frazil ice deposit and the scouring or erosion of microbial communities living on bed or bank surfaces. The DO cycling observed following breakup is quite similar to that observed by White and Melloh (1999) in the relatively pristine Sleeper's River, VT, where biological activity was thought to be minimal.

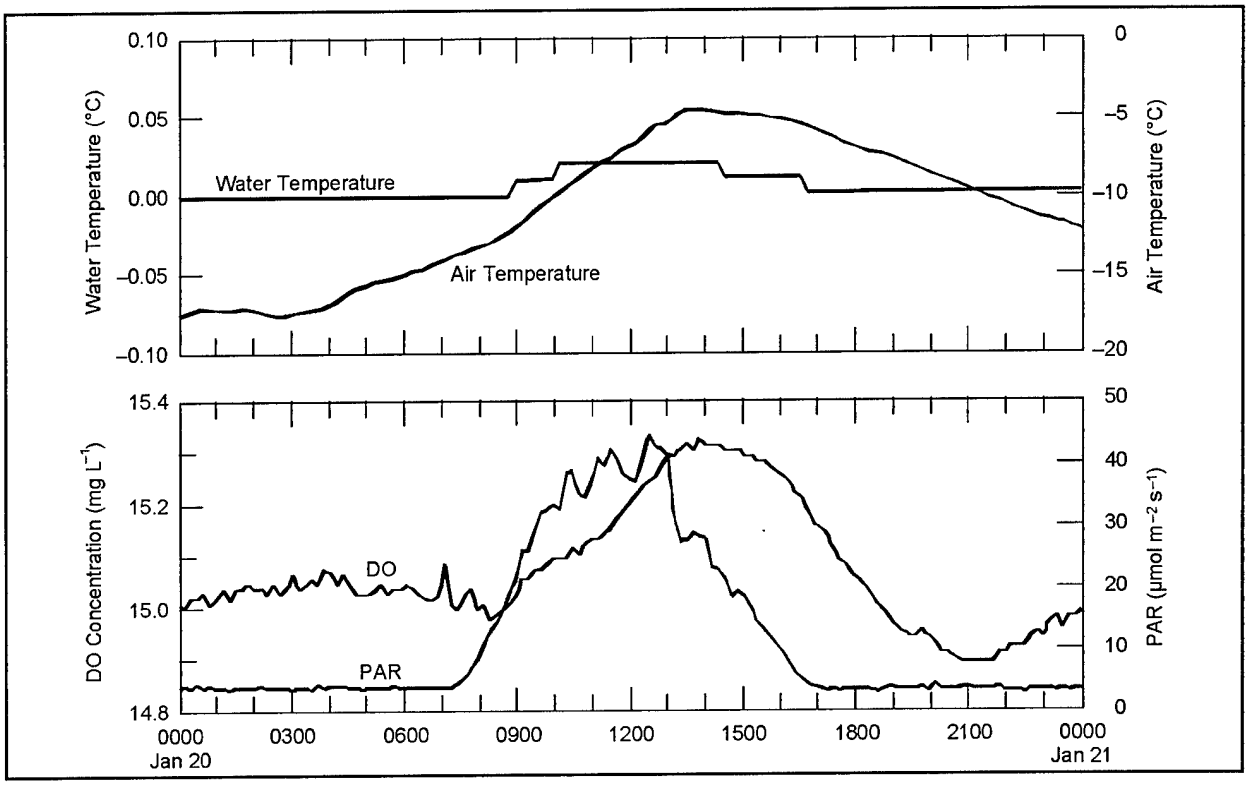

Figure 8. Typical DO, PAR, and temperature data during ice-covered period (20 January 2000). A similar pattern exists in open water during December, January, April, and May

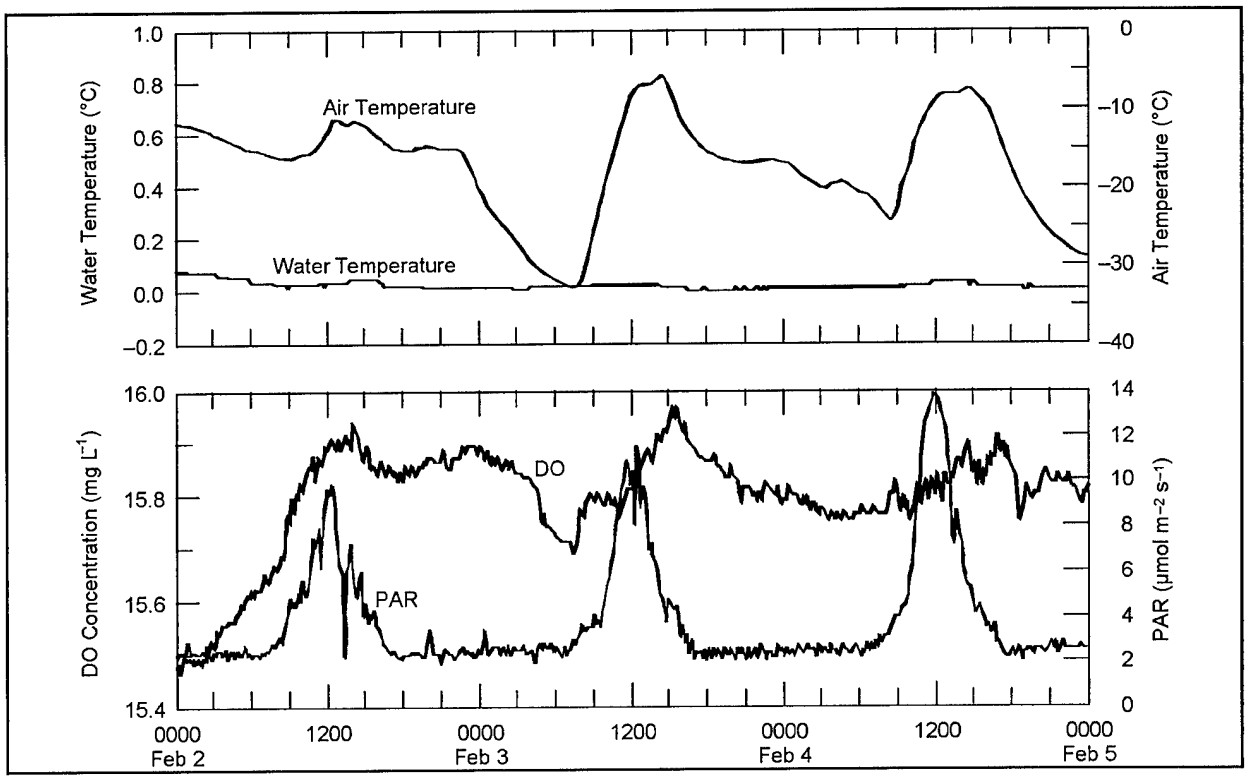

Figure 9. Less well-defined DO cycling occurs during later ice-covered periods, as illustrated for the period 2-4 February 2000

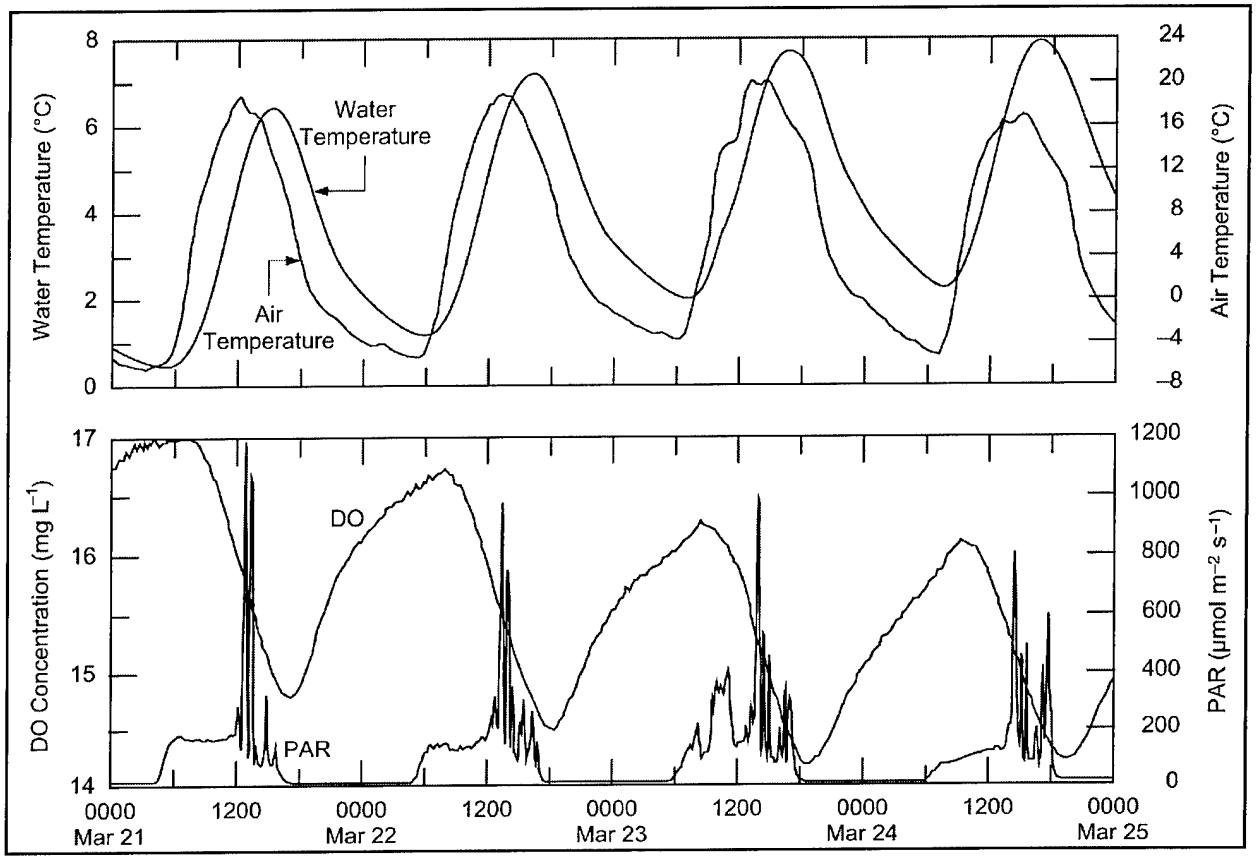

Figure 10. Typical DO, PAR, and temperature data following ice cover breakup (21-24 March 2000)

Respirometer Data. Cumulative evolved CO_2 respiration data obtained from the respirometer study are shown in Figure 11. Water samples collected on 6 and 15 January were evaluated at 1 °C and 25 °C. On 6 January, the river had only intermittent ice cover, but by 15 January the ice cover was approximately 10 cm thick at the sampling site. Both samples had similar respiration patterns at 1 °C. This was shown by a lag period of approximately 100 hr, followed by an increase in respiration from 100 hr to 270 hr to a higher but relatively constant rate. At 1 °C, samples from both 6 and 15 January behaved similarly with respect to CO_2 evolution, each evolving a cumulative total of approximately 50 µl CO_2/ml during the 270-hr incubation, and each having a lag time of approximately 100 hr.

Additional aliquots of the water samples collected on 6 and 15 January were also incubated at 25 °C (Figure 11). Using constant temperature baths on the respirometer, these incubations were conducted concurrently with the 1 °C incubations. Data for both sample collection times again show a lag period of approximately 100 hr. However, from 100 to 270 hr, there was greater CO_2 evolution from the water sample collected on 15 January under ice cover than from the sample collected on 6 January in open water. Cumulative CO_2 evolution values were approximately 330µl CO_2/ml and 170 µl CO_2/ml for the 15 January and 6 January samples, respectively. Because the CO_2 evolution response to warmer incubation temperatures was significantly different between samples collected on 6 and 15 January, these data strongly suggested that there were different microbial communities in the river during open water and ice-covered periods.

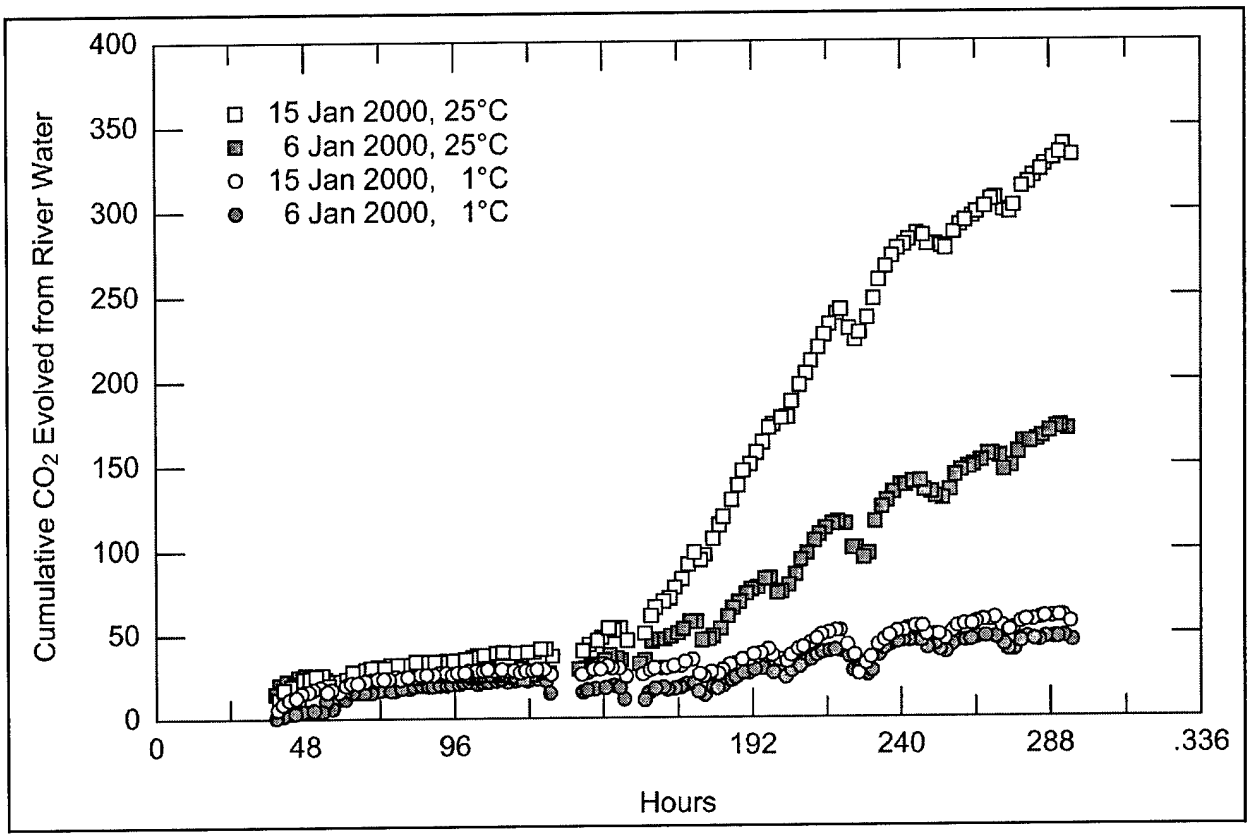

Figure 11. Cumulative respiration data for samples obtained 6 and 15 January 2000

A possible explanation for greater CO_2 evolution from the 15 January early ice-covered sample than from the 6 January open-water sample is that a frazil ice matrix had formed by 15 January, providing structure for biofilm attachment and growth. Visual observations on 15 January revealed algae both within the frazil ice and in accumulations at the surface in open-water areas (Figure 6). Either algae or bacteria present in such a biofilm would supply greater carbon to the system. Because the respirometer has insufficient light to support algal photosynthetic processes in the incubation vessels, catabolic bacterial processes would dominate during respiration studies. Greater release of CO_2 from the ice-cover sample taken on 15 January would support the idea of greater biomass carbon, whether algal, bacterial, or both, in the ice-covered sample relative to the open-water sample.

The high DO measurements are relatively consistent during late ice-covered periods (Figure 9) compared to open water or following ice cover breakup (Figure 10). The DO data suggest either greater DO sources or lesser DO sinks under an ice cover relative to open water. Greater CO_2 evolution from the respirometer data (Figure 11) suggest greater carbon in the water sample taken during ice cover, again probably due to algal biomass contained within the frazil ice matrix.

Microbial Data. In addition to the respiration tests, microbial communities were characterized with a modified fatty acid-based procedure, FAME. This comparison is based on culturable microorganisms that were recovered from the glass-bead samplers and integrates the fatty acid profiles of all culturable microorganisms from a sample into a single profile. In effect, this approach treats an entire sample as an "organism" and then compares the similarity or dissimilarity among samples. Because this approach requires growth on 0.1X TSA, the initial data favors bacterial changes over

fungal or algal changes. These samples represented periods with a well-established ice cover (25 February), immediately following ice breakup (29 February), and over six weeks after ice out (24 April).

The PCA results for the fatty acid data obtained from culturable microorganisms recovered using the glass-bead samplers are shown in Figure 12. Ellipses have been added simply to better show groupings. For the purposes of this study, the importance of these data is that the culturable microbial communities from the river clearly separate into three distinct groups among the three sampling times. These data corroborate the respirometer data and support the concept that there are seasonal, ice-cover-related microbial community structure differences in the river.

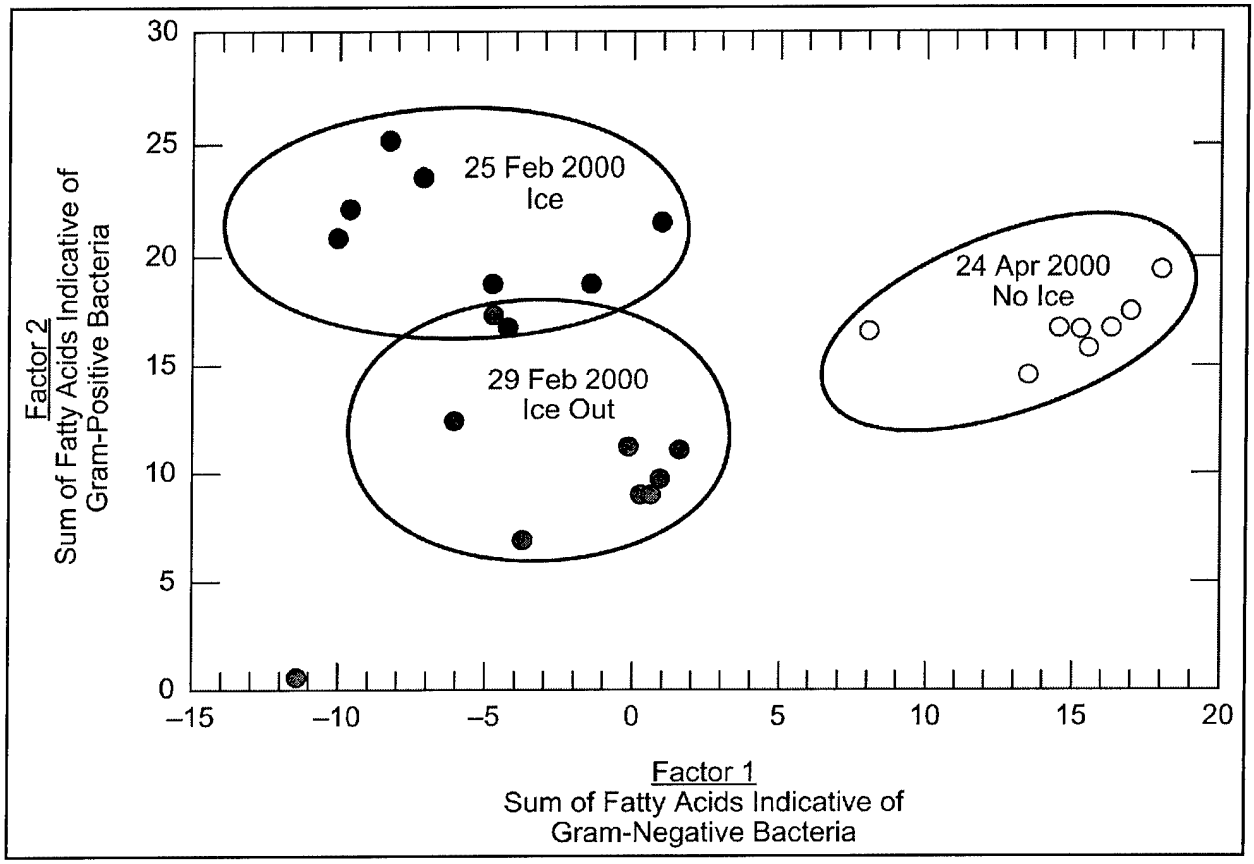

Figure 12. Results of the principal component analysis of fatty acid data obtained from culturable microorganisms collected by the glass bead samplers during the ice-covered period, immediately after ice cover breakup, and well after ice cover breakup

Reaeration Coefficients. The concentration of DO in a river at any time is a function of the deoxygenation resulting from the biochemical oxidation of organic matter in the river and its sediment and the reaeration provided by tributaries, surface runoff, groundwater inflow, photosynthesis, and atmospheric absorption of oxygen (see, e.g., Tchobanoglous and Schroeder (1987)). Freeze and Cherry (1979) note that few measurements of DO in groundwater exist, but that groundwater found in sandy or gravelly soils, or where little soil overlies fractured rock can be oxygenated, while shallow groundwater in silty or clayey soils, similar to those in the project area, is commonly deoxygenated. The component of reaeration associated with atmospheric absorption

depends on the DO saturation level, the DO concentration of the water, the air-water DO concentration gradient, the area of the water surface, and hydraulic factors such as turbulence and dispersion. Its saturation level controls the maximum level of oxygen at the surface of the water, which is a function of temperature and barometric pressure. However, within the water column, the water can become supersaturated by as much as 200 percent (Thomann and Mueller 1987) due to photosynthetic oxygen production.

The DO in a river system is utilized in aerobic respiration as the terminal electron acceptor, in the oxidation of organic and nitrogenous material, and in the oxygen demand exerted in the benthic community. The opposing processes of photosynthesis and respiration often result in a typical diurnal cycle such as that shown in Figure 10. The level of DO in the sediments is related to redox potential (E_h) in sediments (Fletcher 1979), which is generally positive in the well-oxygenated surface made up of larger particles with little organic matter (i.e., favoring oxidation reactions) and negative in the deeper sediments (i.e., favoring reduction reactions) where the particle size is finer and there is more organic matter, thereby affecting the chemical environment (Figure 5). Marchand (1996) notes that if DO drops to very low levels (as in lake bottoms during winter), some organisms switch to anaerobic respiration using glycolysis.

Successful modeling of DO in rivers under open-water and warm-weather conditions has been performed using both simple and complex models. However, it appears that existing deterministic and stochastic models for ice-covered rivers underestimate reaeration during ice-covered conditions (e.g., Landine (1970), Chambers et al. (1993)), do not adequately address the hydraulic effects of the ice cover, and tend to neglect or minimize the importance of ecological effects such as microbial succession during winter. The modeling of DO in rivers was first proposed by Streeter and Phelps in their classic 1925 paper, which presents a one-dimensional, steady-state analytical model to describe the rate of change in DO with time as a function of both biochemical oxidation and reaeration. Based on field observations, Streeter and Phelps assumed that the concentration of DO in a river at any time is a function of deoxygenation and the reaeration provided by the atmospheric absorption of oxygen. Changes in DO due to photosynthesis, sediment oxygen demand, and respiration are neglected, and the biochemical oxygen demand is assumed to decrease monotonically over time:

$$-\frac{dL}{dt} = \frac{dD_d}{dt} = K_d L \tag{2}$$

where t is time in days, D_d is the deoxygenation in mg/l, K_d is the coefficient defining the rate of deoxygenation, and L is the biochemical oxygen demand (also known as the ultimate BOD, or BOD_L). A first-order process is also used to model reaeration:

$$\frac{dD_r}{dt} = -K_r D \tag{3}$$

where D_r is the deoxygenation in mg/l and K_r is the coefficient defining the rate of reaeration. The oxygen saturation deficit D (mg/l) is the sum of the deoxygenation (D_d) and the reaeration (D_r) at any time t, so that the rate of change of the oxygen deficit is a linear, first-order differential equation:

$$\frac{dD}{dt} = \frac{dD_d}{dt} + \frac{dD_r}{dt} = K_d L - K_r D \tag{4}$$

Typically, Equations 2 and 4 are rewritten as a set of coupled first-order equations that describe that change in oxygen deficit with distance:

$$u\frac{dL}{dx} + K_d L = 0 \tag{5}$$

and

$$u\frac{dC}{dx} + K_d L - K_r(C_s - C) = 0 \tag{6}$$

where u is velocity, C is the DO concentration at any point, and C_s is the DO concentration at saturation, which varies with temperature and pressure. The oxygen deficit D is equal to C_s-C. Variations of these equations are commonly used to calculate DO in rivers downstream from wastewater point sources (see, e.g., Tchobanoglous and Schroeder (1987), and Thomann and Mueller (1987)). White (1998) utilized a simple finite element model to represent Equations 5 and 6. This model is modified in the present study to examine diurnal cycling of dissolved oxygen by substituting *dx/dt* for u so that changes in the variables are computed over time rather than distance. Reaeration coefficients can then be examined during different time periods for best fit.

Most models include additional sink and source terms to account for the impacts of photosynthesis, respiration, and other physical processes affecting oxygen levels. However, in practice these sink and source terms are often neglected, with the result that all of the physical processes affecting dissolved oxygen are included in the reaeration and deoxygenation coefficients. Little research has been done regarding appropriate K_r and K_d values at low temperatures in natural rivers, and as Eheart and Park (1989) noted, the temperature adjustment factors commonly used are not well-established. Streeter and Phelps (1925) estimated K_r and K_d for December through February on the Ohio River for water years 1915 through 1917. Their mean values for K_r and K_d of 0.3 d^{-1} and 0.05 d^{-1}, respectively, for two reaches during the winter months were statistically significantly lower than the summer values (White 1998). McBean et al. (1979) neglected K_r when modeling the St. John River (Maine and Canada) in February, and used a value of 0.26 d^{-1} in November. They used a K_d value of 0.064 d^{-1} during both November and February. Pietroniro, Chambers, and Ferguson (1998) used K_r=0.001 d^{-1} for ice-covered reaches of the Athabasca River, following the work of McDonald, Holley, and Goudey (1989), who neglected K_r for the same river.

The present study utilized the observed data for the LaPlatte River to determine coefficients of reaeration and deoxygenation during the ice-covered period. The diurnal cycling of the data clearly showed some relationship between DO and PAR, and hence it seemed reasonable to include the effects of PAR when computing DO. Rather than add a source or sink term, it seemed practical to modify the K_r term, since changes in the level of reaeration would be the primary result of changes in PAR. A simple linear relationship was used to account for these effects:

$$K_{par} = \alpha P_{tl} + K_r \tag{7}$$

where K_{par} is the reaeration coefficient at time t modified to reflect PAR and α is a coefficient describing the relationship between PAR at time $t-l$ (P_{tl}) where l is a specified lag time ranging from 0 for open-water conditions to about 3 hr for late ice-covered period conditions. Because continuous BOD data were not available, BOD was assumed to be constant. Initial values of K_r = 0.3 d^{-1} and K_d = 0.06 d^{-1} were selected. By trial and error, the smallest least-squared error during ice-covered periods was achieved using K_r = 0.7 d^{-1} and K_d = 0.1 d^{-1}, with α = 0.02 and a lag time of 0.12 day. Lag time and K_r decreased for wintertime open-water periods, while K_d increased slightly.

Despite the simplicity of the model and its assumptions, computed DO is quite close to observed DO, and does exhibit diurnal cycling similar to that observed. For example, during the open-water period of 1 to 6 January 2000, DO varied between about 13.1 mg/L and 14.2 mg/L with clear diurnal variations, and averaged 13.7 mg/L. For comparison, the computed DO during the same period ranged from 13.4 to 14.2 mg/L with a mean value of 13.7 mg/L. The computed and observed DO values for this period are shown in Figure 13. Here, K_r = 0.5 d^{-1} and K_d = 0.12 d^{-1}, with α = 0.02 and no lag time. If the relationship shown in Equation 7 is not used, K_r becomes constant and diurnal cycling is not modeled (Figure 13).

During the ice-covered period, smaller diurnal fluctuations in DO were observed. Typical model output for the later ice-covered period, 31 January to 15 February 2000, is shown in Figure 14, with K_r = 0.7 d^{-1}, K_d = 0.1 d^{-1}, a = 0.02, and l = 0.12 day. During this period, DO ranged from 13.0 mg/L to 14.0 mg/L, with a mean value of 13.6 mg/L. The low levels of observed PAR prior to 9 February (daily maximums less than 15 $\mu mol/m^2$ s) induce small yet discernible changes in computed diurnal cycling of DO. Increased levels of PAR on 10, 12, 13, and 15 February result in increased levels of computed DO for those days. The relatively large increases in observed DO on 8 and 13-14 February are more closely correlated with decreases in air temperature than changes in PAR, and thus the model slightly underestimates DO on these days. Computed DO for the period 31 January to 15 February 2000 ranged from 13.1 mg/L to 13.8 mg/L with a mean value of 13.6 mg/L. However, the error is much less than if constant K_r = 0.7 d^{-1} is used (Figure 14), which ranged from 13.1 mg/L to 14.3 mg/L with a mean value of 13.9 mg/L.

CONCLUSIONS AND RECOMMENDATIONS: The purpose of this study was to investigate riverine dissolved oxygen concentration during winter, including the ice-covered period, with emphasis on microbial processes contributing to reaeration and deoxygenation. Data showed that DO patterns in a small Vermont river are different during an ice-covered period than during

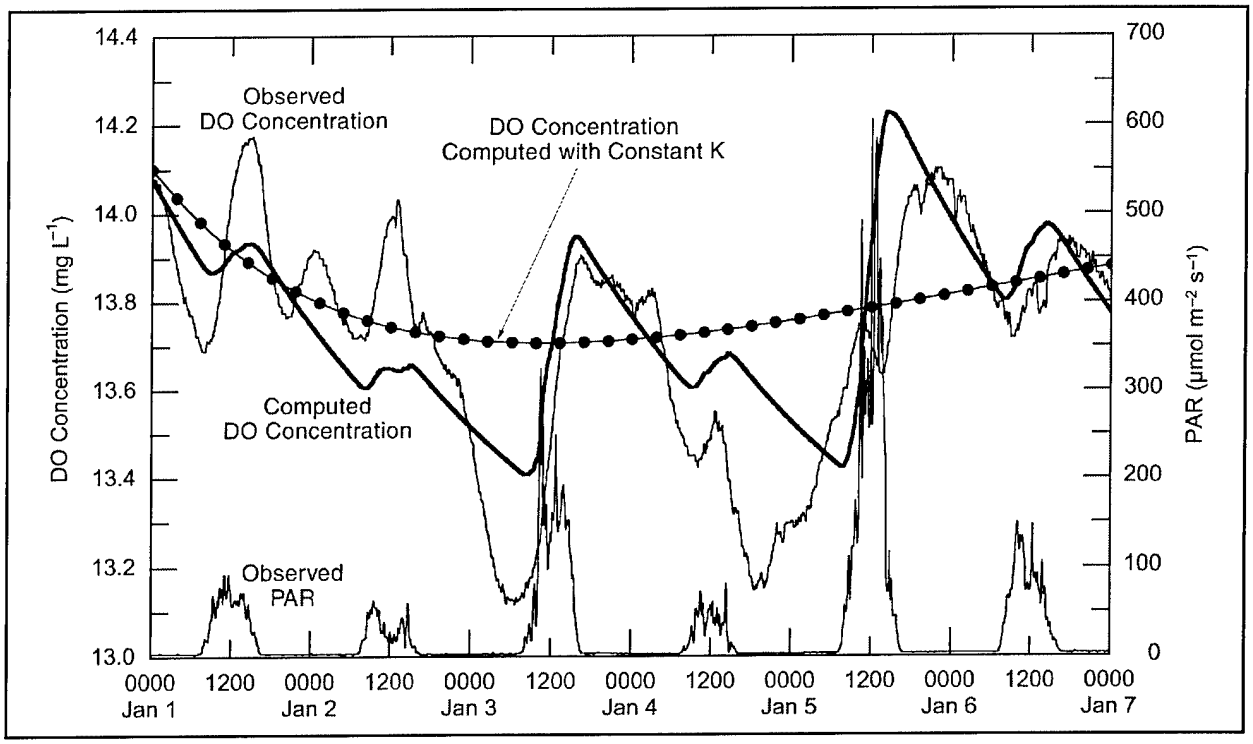

Figure 13. Observed versus computed DO for the open-water period 1-6 January 2000. For comparison, DO computed using constant $K_r = 0.5\ d^{-1}$ is also shown. Observed PAR is shown for the same period

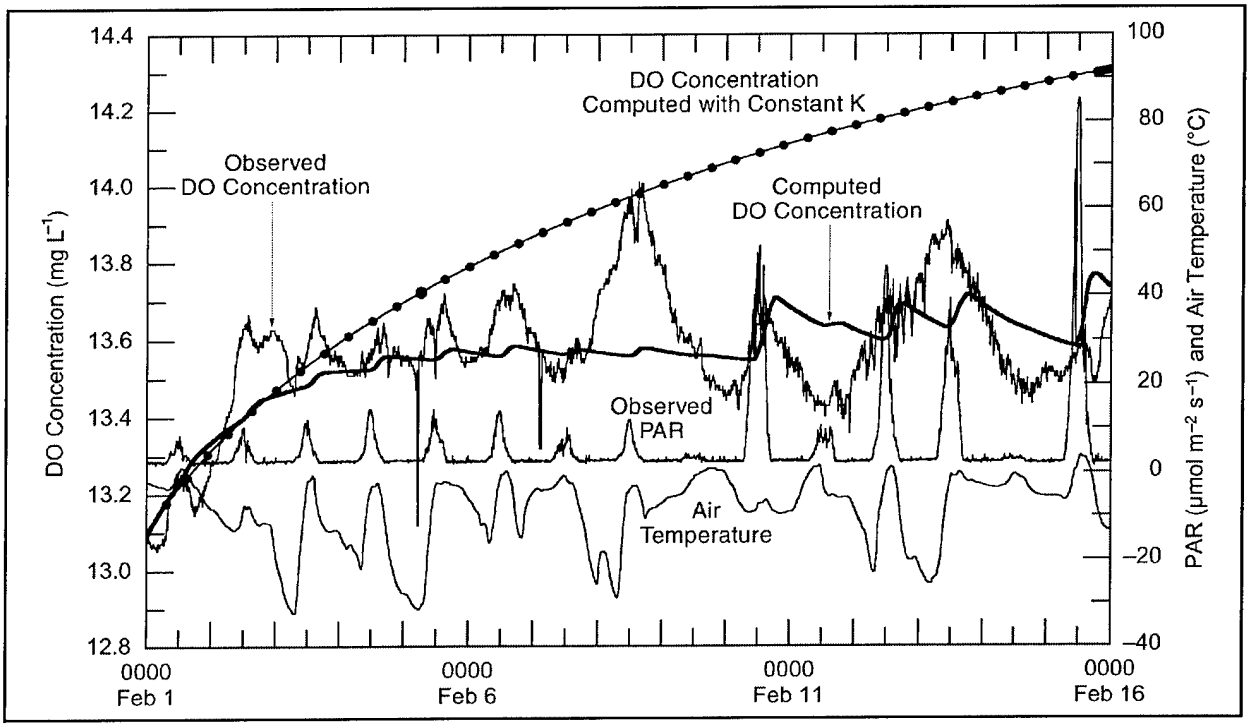

Figure 14. Observed versus computed DO and observed PAR and air temperature during the late ice-covered period 1-15 February 2000. For comparison, DO computed using constant $K_r = 0.7\ d^{-1}$ is also shown

open-water periods. In addition, diurnal cycling of DO persists during the ice-covered period, and appears to be related to PAR in a manner that suggests photosynthetic activity may be contributing to reaeration. Visual observations at the time of ice cover formation showed algae present in the ice cover and within the frazil matrix trapped beneath the ice cover. It is possible that the porous frazil structure provides a surface for biofilm formation that supports microbial communities in a different manner than the typical lotic environment. Microbial communities were found by two independent measures to be different under an ice cover than in open water.

Previous modelers have either neglected reaeration during winter or have used very small values for the reaeration coefficient K_r. Data showed DO levels to be at or above saturation levels for the ice-covered period, indicating that reaeration could be important. A simple finite element model based on the classic Streeter-Phelps model was applied to the field data to evaluate reaeration and deoxygenation coefficients. The use of a simple linear relationship in the model between PAR and K_r to account for diurnal cycling greatly reduced error compared to using a constant value of K_r. Application of the model to winter open-water and ice-covered periods indicates that both K_r and K_d are higher than previously thought. Results show K_r is not negligible, and probably lies between about 0.5 and 0.75, while K_d is on the order of 0.1, not substantially different than values used by others.

The results of this study indicate that photosynthetic activity can be important in winter water quality. The presence of an ice cover does not prevent photosynthetic activity. These data support the concept that improved understanding of the interactions among frazil ice, ice-covered water, and the microbiology of ice and water may suggest ways to improve winter water quality. The use of phospholipid ester-linked fatty acid analysis of samples extracted directly from water may avoid problems with culturing. Water samples should be obtained within and below the frazil accumulation in order to more clearly identify potential community differences. Additional field data are required to test and refine the model, including the effects of air temperature, and to develop a range of reaeration and deoxygenation coefficients useful to modelers.

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