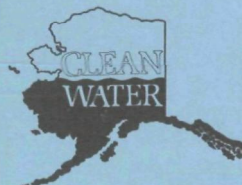

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LABORATORY EVALUATION OF AN IMPROVED SAMPLING PROCEDURE FOR DISSOLVED OXYGEN



**ENVIRONMENTAL PROTECTION AGENCY
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RESEARCH CENTER**

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LABORATORY EVALUATION OF AN IMPROVED SAMPLING PROCEDURE
FOR DISSOLVED OXYGEN IN STREAMS IN EXTREMELY COLD CLIMATES

by

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A Working Paper presents results or investigations
which are to some extent limited or incomplete.
Therefore, conclusions or recommendations--
expressed or implied--are tentative.

INTRODUCTION

Oxygen dissolved in stream waters is essential to most forms of aquatic life. Under some environmental conditions natural concentrations may be less than optimum. This is especially true for some Alaskan streams in winter when photosynthetic activity is reduced to insignificance by thick ice cover and low to zero light intensity caused by short daylight periods and low solar angles. Even when water temperature remains at 0°C some oxygen is required for respiration processes by most aquatic organisms. In Alaska it has been documented that, under the prolonged ice cover caused by long winters, dissolved oxygen (DO) may be reduced to less than 2 mg/l (Frey, 1969; Frey, Mueller, and Berry, 1970). Such low concentrations are well below the level of 5 mg/l recommended by the Committee for Water Quality Criteria (1968) for supporting most aquatic life, and any additional oxygen demand caused by pollutants could be disastrous to aquatic communities. The natural occurrence of such low concentrations becomes especially relevant when establishing water quality standards.

Because of this apparent natural occurrence of depressed DO concentrations in waters of Interior Alaska, a field study was designed and completed in the winter of 1970-71 to measure dissolved oxygen in some representative streams (Schallock and Lotspeich, 1972). Data gathered by this study verified that levels may range from near zero to saturation even during a prolonged cold winter. Moreover, because of very cold temperatures, standard sampling procedures cannot successfully be used and filling unstoppered sample bottles by immersion with turbulent displacement of air (or other gas) may be required. It was suspected that at these low concentrations, errors inherent in the method might become significant.

Because of this suspected error, a new technique of utilizing a nitrogen-filled sample bottle was designed by Schallock (1972). Comparison of nitrogen-filled bottles with air-filled bottles was made when measuring low DO during winter field conditions. Results of this work clearly established that DO concentrations sampled by displacing nitrogen were consistently and significantly lower than by air displacement. However, these data did not establish which method was more accurate. The project described here was designed as a laboratory study to determine which method is the more accurate and to elucidate on the causes of differences in determinations between nitrogen and air displacement.

PROCEDURE

A synthetic-rubber coated 55-gallon drum filled with tap water was placed in a cold room maintained at a temperature of 0°C. A small stirrer was mounted on the edge of the drum and allowed to run continuously to maintain gentle stirring. The drum was covered with a piece of clear, rigid plastic to prevent drops from splashing out during purging and to reduce evaporation. Nitrogen or oxygen gas was bubbled through the water in the drum to regulate the concentration of dissolved oxygen at any predetermined level. Gases entered the room through plastic tubing from cylinders placed outside, and were introduced into the water at the bottom of the drum through two sintered glass diffusers. A Yellow Springs Instrument Co. (YSI) DO sensor was used to monitor the DO of the water during bubbling by nitrogen or oxygen. A YSI DO meter was mounted outside the cold room to allow it to remain at normal room temperature during DO measurements.

It was noted during initial removal of DO by nitrogen bubbling that at high DO levels, nitrogen effectively removed DO in a short time (within minutes) at small flow rates. As the concentration became lower (less than about 5 mg/l) removal took more time and higher nitrogen flow rates. At the lowest DO level (near 0.1 mg/l) strong turbulence was required to remove oxygen.

Three techniques were used to fill 300 ml BOD bottles: (1) a siphon similar to that described by Magnuson and Stuntz (1970), (2) a clear plastic Van Dorn bottle, and (3) immersion of the bottles mouth side up; removing stoppers after immersion. All sampling was performed in triplicate. Before sampling, bottles were filled with argon or

nitrogen from compressed gas cylinders through a tube held at the bottom of the bottle for 0.5 minutes. After filling with gas, bottles were stoppered with ground glass stoppers. In addition, air-filled bottles were also used. A standard sequence of sampling was maintained: first the siphon, with least disturbance; then the Van Dorn bottle; and last immersion. After sampling was completed, six bottles--three filled with nitrogen and three with air--were again filled by the siphon method to determine the amount by which DO was increased owing to disturbance caused by sample collection. Argon was not used at this step because preliminary interpretation of data indicated that argon and nitrogen gave nearly identical results.

After dissolved oxygen sampling was complete, a 500 ml sample was collected for pH, alkalinity, and conductivity; these measurements were completed within 10 minutes to eliminate changes caused by increased temperature. All water removed during sampling was replaced with similar water from an 8 liter container kept at the same temperature.

Immediately after samples were collected DO was fixed using the azide modification of the Winkler method (Standard Methods, 1971, pp.477-481). For very low concentration of DO (<1 mg/l) the titrant was diluted by a factor of 10 to maintain volumetric accuracy; i.e., the titrant was approximately 0.0025N instead of 0.025N. Triplicate values for each sample were arithmetically averaged. Statistical tests for significance were applied using the t-test for related measures (Bruning and Hintz, 1968, pp. 12-15) which determines the significance of differences between two correlated means.

RESULTS AND DISCUSSION

Although both nitrogen and argon were used as displaced gases, results derived from each were nearly identical and differences in averaged triplicate values between the two were small (usually <0.05 mg/l, only 3 values showing differences of 0.1 mg/l out of 84 groups) and may be considered insignificant compared to consistently greater differences between these gases and air. The t-test (Table 1) showed that differences in the behavior of these gases were not significant at the 80% confidence level for the siphon method. Both behaved similarly even though argon is about 1.5 times as dense as nitrogen; these comparisons lead us to conclude that results when using nitrogen may be valid and this gas may not be removing significant amounts of DO from the water during sample collection except at high DO concentrations.

TABLE 1

T-Test for Related Measures to Determine the Significance of Difference
Between Two Correlated Means (Bruning and Hintz, 1968)

Sampling Method	Comparing	t _{RM}	T-Values for a Range of Confidence Levels
siphon	N ₂ vs air	2.44349	T(0.001) = 4.587
	N ₂ vs Ar	0.62988	T(0.01) = 3.169
	air vs Ar	2.95716	T(0.02) = 2.764
nitrogen filled	siphon vs Van Dorn	2.58995	T(0.05) = 2.228
	siphon vs immersion	1.23951	T(0.10) = 1.812
	Van Dorn vs immersion	2.17057	T(0.20) = 0.700
immer-sion	N ₂ vs air	19.19287	
	N ₂ vs Ar	1.07853	
	air vs Ar	19.00418	

The close similarity of data using nitrogen and argon becomes apparent when compared with air using the immersion method (Figure 1). Data for siphon and Van Dorn methods are so similar that they are not displayed graphically but are recorded in tables to be discussed later. From Figure 1 it is quite apparent that DO measured when air is the displaced gas is consistently about 0.3 to 0.5 mg/l higher than for either of the other two gases. The t-test was applied to compare argon with air, nitrogen with air, and nitrogen with argon using the immersion method. Differences between air and argon or nitrogen were highly significant at the 99% confidence level; however, differences between argon and nitrogen were not significant at the 90% level level. At DO concentrations above that normally found in streams without an ice cover this introduces errors ranging from about 10% at 5 mg/l to 3% near saturation at 0.0°C. Below 5 mg/l errors in the air immersion method increase to about 300% at the concentrations measured in several unpolluted streams of Interior Alaska during winter (Frey, 1969, Frey et al. 1970). Thus it becomes extremely important that there be a more accurate sampling technique for DO at these low concentrations. A final bar graph comparing these gases using the siphon method is shown in Figure 1; these differences are very small at supersaturation. Only the immersion method, with its attendant turbulence, appears to be inaccurate.

Data comparing sampling methods, using air as the displaced gas, are given in Figure 2, and show that immersion gives a consistent error at DO concentrations less than about 9 mg/l. Above this concentration all three methods produced varying results, near or above saturation the immersion method gave low values. At low DO concentrations the immersion method evidently introduces oxygen from the displaced air to the water.

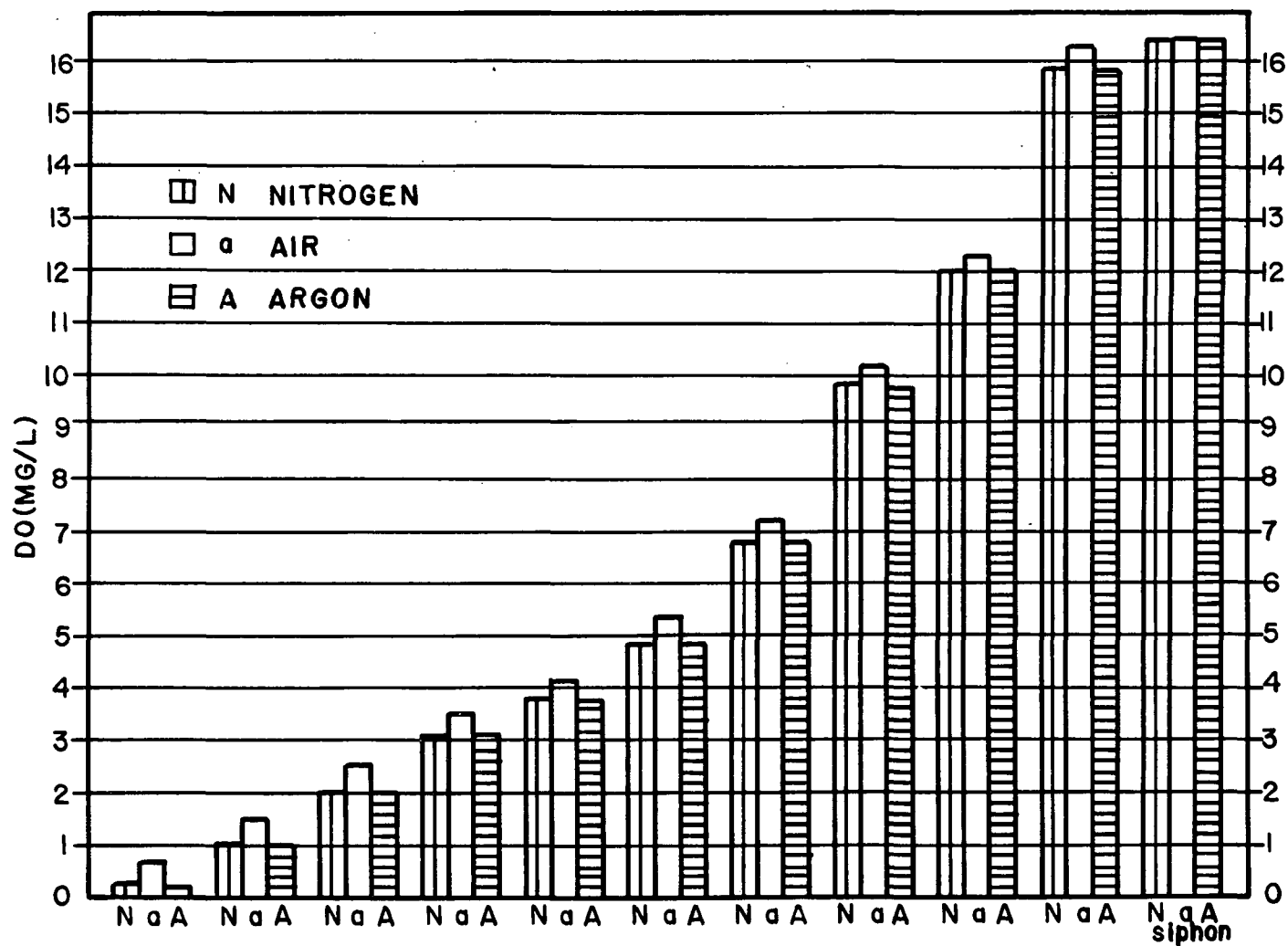


Figure 1. Comparing dissolved oxygen in tap water (sampled by immersion) using air, nitrogen, and argon as the displaced gases. One siphon sampling at the highest DO level is included to show how all gases behave with non-turbulent sampling.

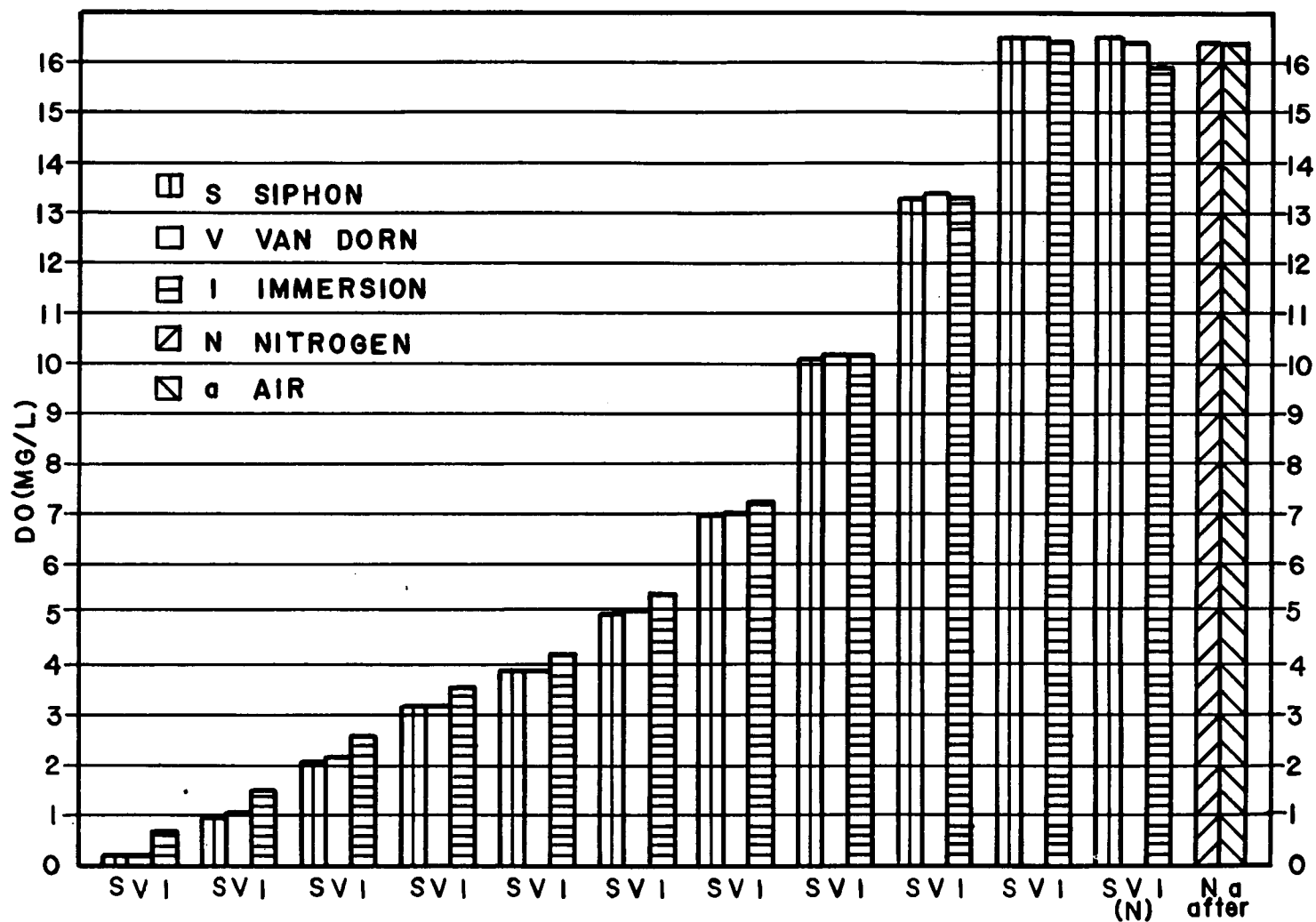


Figure 2. Comparing dissolved oxygen in tap water (using air as the displaced gas) using siphon, Van Dorn, and immersion sampling procedures. One additional run using nitrogen as the displaced gas is included to compare sampling methods at high DO concentrations.

This tendency decreases as DO levels approach saturation, where turbulence may remove some DO. The bars for nitrogen in Figure 2 indicate that immersion actually strips oxygen from the water; although not graphed, similar results were found for argon. Siphon samples taken after all techniques were completed are also shown as the final bars and closely approach DO levels taken with minimum disturbance. This is interpreted as indicating that little oxygen was introduced or removed from the container by disturbance from sampling by all three methods.

Table 2 presents complete data from tap water and suggests that a positive error is introduced by sampling with the Van Dorn bottle; this error increases at high DO. This is interpreted as being caused by the greater disturbance of Van Dorn sampling compared to the very quiet siphon. Other parameters measured include pH, alkalinity, and specific conductance; of these, only pH shifted significantly as DO was lowered. Stripping with bubbling nitrogen evidently also strips out carbon dioxide, causing an increase in pH as CO_2 is removed. This removal of dissolved gases by stripping does not simulate stream dynamics and under natural conditions pH is not necessarily increased at extremely low DO. In addition to the above observations, this table shows the close agreement of DO measurement using argon and nitrogen in sampling bottles by each of the three methods. At low DO concentrations (<about 5 mg/l) all sampling methods gave values within about 0.1 mg/l. Also illustrated are the significant differences when comparing air-filled bottles with argon-filled bottles at low concentrations of DO; another observation is the close agreement in values between air- and nitrogen-filled bottles using the siphon technique from water sampled after the disturbance caused by sampling to test differences among gases. The

TABLE 2

Dissolved Oxygen Sampling Evaluation (Tap Water)

Gas Dis-placed	Dissolved Oxygen (mg/l) Sampling Method				Other Parameters			
	Siphon	Van Dorn	Dunking	Siphon After	Temp. °C	Cond. umoh/cm	pH	Alk. mg/l
N ₂	16.47	16.40	15.88	16.41				
air	16.50	16.51	16.37	16.44	0.8	500	-	78
A	16.47	16.39	15.91					
N ₂	13.28	13.32	12.97	12.93				
air	13.32	13.37	13.30		-	480	8.05	85
A	13.34	13.29	13.01					
N ₂	10.11	10.13	9.85					
air	10.13	10.20	10.18	10.09	0.3	500	7.85	86
A	10.05	10.09	9.78					
N ₂	6.96	7.01	6.82					
air	6.99	7.01	7.26	7.03	0.3	500	8.10	86.5
A	6.92	6.96	6.81					
N ₂	4.95	5.05	4.87	5.02				
air	5.01	5.11	5.40	5.08	1.0	500	8.00	86.0
A	4.96	4.99	4.84					
N ₂	3.85	3.96	3.84	3.93				
air	3.93	3.88	4.21	3.92	0.2	500	8.00	85.0
A	3.81	-	3.80					
N ₂	3.15	3.25	3.17	3.24				
air	3.22	3.23	3.54	3.25	0.3	500	8.30	84.5
A	3.11	3.25	3.14					
N ₂	2.00	2.09	2.08	2.11				
air	2.10	2.21	2.63	2.12	0.8	500	8.60	81.0
A	1.98	2.03	2.08					
N ₂	1.97	2.05	2.03	2.05				
air	2.03	2.13	2.53	2.13	0.3	500	8.30	84.5
A	1.95	2.06	2.02					
N ₂	0.93	0.97	1.04	1.02				
air	0.98	1.07	1.52	1.03	0.2	500	8.60	87.0
A	0.94	1.00	1.05					
N ₂	0.11	0.23	0.27	0.20				
air	0.20	0.20	0.70	0.24	0.8	500	8.6	81.0
A	0.20	0.23	0.22					

t-test (Table 1) was applied to the differences among sampling techniques using nitrogen as the displaced gas. These tests indicate that differences between the siphon and Van Dorn bottle are insignificant above the 95% confidence level, for Van Dorn and immersion above the 90% level, and siphon versus immersion above the 80% level; correlation between any two methods was consistently above 0.999. This is interpreted as indicating that all three methods will give reliable DO results when nitrogen is used as displaced gas.

Data derived in identical sampling procedures are shown in Table 3 for Chena River water collected above 80 river miles above Fairbanks. Although alkalinity and specific conductance are significantly less than for the tap water used in the earlier run, results and conclusions are similar. This suggests that chemical properties of water do not significantly influence the sampling of DO, at least at the concentrations measured in these two waters.

In its lower reaches, Chena River receives domestic waste waters from primary treatment plants from Fort Wainwright and Fairbanks. This limited treatment results in a pollution load many times higher than that present in reaches upstream. To test the possible effects of this pollution on sampling methods and avoid possible criticism that BOD from pollution might influence DO mechanisms in our laboratory procedure, 100 gallons of river water from below Fairbanks was brought into the laboratory and treated in the same manner as that described earlier. Data generated using this water are recorded in Table 4. Despite the distance separating these sampling stations (about 80 to 100 river miles) with two major tributaries entering between these stations and the fact

TABLE 3

Dissolved Oxygen Sampling Evaluation (Upper Chena River)

Gas Dis-placed	Dissolved Oxygen (mg/l) Sampling Method				Other Parameters			
	Siphon	Van Dorn	Dunking	Siphon After	Temp. °C	Cond. umoh/cm	pH	Atk. mg/l
N ₂	17.62	17.62	17.11	17.57				
air	17.70	17.59	17.64	17.67	0.6	200	7.80	57.0
A	17.63	17.57	17.08					
N ₂	14.00	13.96	13.53	13.92				
air	14.04	14.03	14.02	13.91	0.6	200	7.80	57
A	13.96	14.93	13.48					
N ₂	12.50	12.48	12.10	12.39				
air	12.55	12.52	12.56	12.46	0.5	200	7.70	56.5
A	12.46	12.56	12.07					
N ₂	10.09	10.04	9.80	10.04				
air	10.12	10.14	10.22	10.10	1.2	210	7.75	57.0
A	10.07	10.02	9.76					
N ₂	8.19	8.20	7.97	8.23				
air	8.26	8.29	8.50	8.26	0.5	210	7.90	56.5
A	8.17	8.16	7.98					
N ₂	5.81	5.83	5.72	5.80				
air	5.84	5.91	6.20	5.91	0.4	200	7.85	57.0
A	5.77	5.80	5.68					
N ₂	3.56	3.63	3.57	3.61				
air	3.60	3.72	4.03	3.70	0.9	210	7.90	57.0
A	3.54	3.59	3.52					
N ₂	2.11	2.16	2.14	2.18				
air	2.13	2.24	2.63	2.21	0.9	200	8.15	57.0
A	2.10	2.18	2.13					
N ₂	0.16	0.24	0.36	0.26				
air	0.23	0.37	0.72	0.33	1.0	200	8.40	57.0
A	0.17	0.24	0.26					

TABLE 4
Laboratory Study of DO (Lower Chena)

Gas Dis- placed	Dissolved Oxygen (mg/l) Sampling Method				Other Parameters			
	Siphon	Van Dorn	Dunking	Siphon After	Temp. °C	Cond. umoh/cm	pH	Alk. mg/l
N ₂	13.37	13.38	12.99	13.30				
air	13.50	13.44	13.46	13.41	0.2	200	7.8	72.5
A	13.36	13.37	13.09					
N ₂	16.89	16.90	16.38	16.77				
air	16.97	16.99	16.83	16.85	0.1	200	7.8	71
A	16.89	16.84	16.32					
N ₂	10.69	10.73	10.44	10.70				
air	10.79	10.79	10.52	10.78	0.2	190	7.8	71
A	10.71	10.72	10.37					
N ₂	6.80	6.83	6.70	6.85				
air	6.89	6.93	6.62	6.89	0.1	180	7.7	69
A	6.81	6.85	6.66					
N ₂	5.04	5.11	4.99	5.08				
air	5.13	5.17	5.46	5.16	1.1	190	8.0	69
A	5.05	5.14	4.96					
N ₂	2.87	2.92	2.89	2.96				
air	2.96	3.09	3.45	3.08	1.1	185	8.0	69
A	2.85	2.99	2.90					
N ₂	1.19	1.38	1.26	1.28				
air	1.27	1.33	1.73	1.35	0.5	185	8.3	69
A	1.15	1.48	1.30					
N ₂	0.43	0.50	0.60	0.51				
air	0.47	0.70	1.12	0.63	1.2	185	8.4	69
A	0.39	0.56	0.56					

that one site is below a seriously polluting source, data in Table 4 closely resembles that in Table 3. There are no significant differences in the behavior of oxygen in these sampling methods between the two waters.

In sampling by the siphon and Van Dorn bottle, the recommended procedure is to allow 1 to 2 volumes of water to overflow by displacing water from the bottom of the sample bottle. This procedure is usually impossible in winter because of the intense cold that may freeze samples in less than one minute. To evaluate the error that might result from not following this procedure, one and two volumes of water were displaced using the siphon method and tap water. Applying the t-test to this procedure showed that differences between filling and overflowing one and two volumes were not significant at the 90% confidence level for either air or nitrogen. For nitrogen, the differences were not significant at the 80% confidence level. From the test of these data it may be concluded that any error introduced by not overflowing (which is impossible by the immersion procedure) is insignificant.

CONCLUSIONS

Dissolved oxygen concentrations measured in water samples at 0°C temperature, collected by immersion with air as the displaced gas, are significantly higher at low concentrations than when another gas is displaced. This leads to the conclusion that at very cold temperatures, where immersion must be used, nitrogen or other gas-filled sample bottles should be used. At DO levels near saturation and above, both nitrogen and argon give low values, evidently causing by stripping of easily removed oxygen at these concentrations.

Quiet displacement of any gas is preferable to the turbulence caused by immersion. Thus, if the siphon technique could be adapted to very cold temperatures, thick ice, and varying water levels, it would be the preferred method. However, results using nitrogen-filled bottles with the immersion sampling method are comparable to those derived by alternate recommended procedures which may not be feasible under extreme winter cold. The immersion method is easily adapted to a wide range of transportation modes; it has been used with a pickup truck when the temperature was -44°C and fixed wing airplane at temperatures as low as -30°C. It is doubtful if other methods would prove as satisfactory under these conditions. Finally, some disturbance of the water surface during sampling does not appear to significantly increase the oxygen content of the water, even at very low DO concentrations.

Results acquired during the course of this investigation appear independent of sample source. One, tap water, is a treated ground water and the other two were from the Chena River, one from about 75 river miles above Fairbanks--unpolluted--and the other about five miles downstream from Fairbanks and strongly polluted. This suggests that, within

the range of conditions encountered on these waters, dissolved oxygen can be measured using the same techniques without special precautionary measures.

Of the three chemical parameters measured only pH appeared to undergo a significant change. This change only occurred at very low DO concentrations where pH increases about 0.5 units. This increase may be the effect of removing CO_2 by N_2 scrubbing and is not believed to be directly related to DO concentrations at these low concentrations.

Elimination of the overflow portion of recommended procedures (extremely difficult under our climatic conditions) does not introduce a serious inaccuracy with this procedure and can be neglected in evaluating field data acquired under winter conditions.

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