

Article

Evaluating a fast headspace method for measuring DIC and subsequent calculation of $p\text{CO}_2$ in freshwater systems

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Abstract

A variety of different sampling and analysis methods are found in the literature for determining carbon dioxide (CO_2) in freshwaters, methods that rarely have been evaluated or compared. Here we present an evaluation of an acidified headspace method (AHS) in which the dissolved inorganic carbon (DIC) is measured from an acidified sample and the partial pressure ($p\text{CO}_2$) is calculated from DIC using pH and water temperature. We include information on practical sampling, accuracy, and precision of the DIC/ $p\text{CO}_2$ determination and a storage test of samples. The $p\text{CO}_2$ determined from the AHS method is compared to that obtained from the more widely used direct headspace method (DHS) in which CO_2 is equilibrated between the water and gas phases at ambient pH. The method was tested under both controlled laboratory conditions as well as wintertime field sampling. The accuracy of the DIC detection was on average 99% based on prepared standard solutions. The $p\text{CO}_2$ determination in lab, using the DHS method as a reference, showed no significant difference, although the discrepancy between the methods was larger in samples with $<1000 \mu\text{atm}$. The precision of the $p\text{CO}_2$ determination was on average $\pm 4.3\%$, which was slightly better than the DHS method ($\pm 6.7\%$). In the field, the AHS method determined on average 10% higher $p\text{CO}_2$ than the DHS method, which was explained by the extreme winter conditions (below -20°C) at sampling that affected the sampling procedure of the DHS method. Although samples were acidified to pH 2, respiration processes were still occurring (at a low rate), and we recommend that analyses are conducted within 3 days from sampling. The AHS method was found to be a robust method to determine DIC and $p\text{CO}_2$ in acidic to pH-neutral freshwater systems. The simple and quick sampling procedure makes the method suitable for time-limited sampling campaigns and sampling in cold climate.

Key words: carbon dioxide, comparison, freshwater, headspace, methods, samples

Introduction

Interest in inland waters and their role in the global carbon (C) cycle has increased rapidly during recent decades. On the global scale, streams, lakes, and other inland waters are now concluded to be significant sources of carbon dioxide (CO_2) to the atmosphere because surface water CO_2 concentrations often exceed atmospheric equilibrium (Richey et al. 2002, Battin et al. 2008, Aufdenkampe et al.

2011). Improved knowledge of the strong connectivity and interplay between terrestrial and aquatic processes, in combination with the recognition that inland waters are active conduits for C transfer over the land–water–atmosphere interfaces, has further increased the awareness of inland water C dynamics (Cole et al. 2007, Tranvik et al. 2009).

The accurate determination of dissolved inorganic carbon (DIC) and CO_2 is a crucial part of the study of C

dynamics in aquatic systems. The carbonate equilibrium is complex, with different C constituents of CO_2 , bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) in both gaseous and dissolved phases, which makes the determination challenging. Several manual methods for determining CO_2 in surface waters are described in the literature, including direct headspace methods (DHS; Kling et al. 1991, Cole et al. 1994, Hope et al. 1995, 2001) and indirect methods that could be mainly divided into acidified headspace methods (AHS; Stainton 1973, Nilsson et al. 2008, Wallin et al. 2010) and titration-based methods (Neal 1988a, Neal et al. 1998, Worrall and Lancaster 2005).

DHS methods are closed systems in which sampled water is equilibrated with a headspace of ambient air or a CO_2 -free gas such as nitrogen (N_2). The samples are vigorously shaken prior to isolation of the headspace, followed by analysis. AHS methods are similar to direct methods in terms of using closed systems evacuated of CO_2 , but with the difference that the sampled water is acidified to shift the carbonate equilibrium toward CO_2 and to inhibit microbial degradation of organic C in the sample.

The analysis of CO_2 in the headspace is then a measure of DIC and requires calculations that account for *in situ* pH and temperature to calculate the partial pressure of CO_2 ($p\text{CO}_2$) in the field. Indirect titration based methods rely on an alkalinity determination, often in open systems where CO_2 can be degassed from the sample during the sampling and analysis procedures. As in the AHS method, these require in-stream pH and temperature in the subsequent procedures to calculate $p\text{CO}_2$.

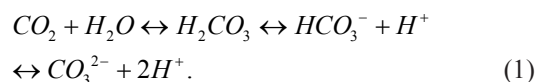
In the absence of large-scale datasets based on headspace methods, recent regional and national estimates of freshwater CO_2 have been indirectly determined by different approaches using measures of dissolved organic carbon (DOC), alkalinity, and pH (Erlandsson et al. 2008, Humborg et al. 2010, Butman and Raymond 2011, Weyhenmeyer et al. 2012). In addition to the manual methods, various techniques to measure $p\text{CO}_2$ continuously in surface waters have been used during the last decades (Carignan 1998, Hari et al. 2008, Johnson et al. 2010).

All methods for measuring and estimating $p\text{CO}_2$ in surface waters have practical and chemical limitations. For instance, the indirect titration approach based on chemical equilibria and charge balances especially has limitations when used for boreal regions where stream pH is often <5.6 and with no alkalinity (Bishop et al. 2008, Wallin et al. 2014). The number of studies that compare methods is limited, but one study by Hope et al. (1995) compared a DHS method with an indirect, titration-based method (Neal 1988a, 1988b) and concluded that the DHS

method generally produced higher $p\text{CO}_2$ than the titration method. This finding was partly explained by degassing of CO_2 from the sample between sampling and analysis. Furthermore, the degassing of CO_2 changed the pH of the sample used in the calculation procedure for the titration-based method. In contrast, Hunt et al. (2011) concluded that $p\text{CO}_2$ was overestimated (13–66%) if calculated from total alkalinity compared with values calculated from DIC due to the influence of organic acids on the alkalinity determination.

In this study we evaluate a published AHS method for DIC determination and subsequent $p\text{CO}_2$ calculation (Öquist et al. 2009, Wallin et al. 2010, 2013) to determine its accuracy, precision, and limitations. We compared the published method with a well-established DHS method used as a reference (Hesslein et al. 1991, Cole et al. 1994, Kelly et al. 2001, Jonsson et al. 2003). In the comparison we use data collected both in the laboratory and in the field.

The AHS method for DIC determination and subsequent $p\text{CO}_2$ calculation can briefly be described as follows. In the field, sample water with a known pH is injected into small sealed and evacuated glass vials (22 mL) prefilled with acid, which lowers the pH, forces all DIC into CO_2 (simplified and described in equation 1; Wetzel 2001), and inhibits most respiration processes:



In the laboratory, a gas chromatograph (GC) connected to an auto sampler facilitates rapid analysis of the headspace CO_2 in a large number of vials. The CO_2 concentration measured in the headspace is then recalculated to DIC because the lowered pH (~ 2) drives the DIC into CO_2 with a partitioning between the water and gas phases according to Henry's law. Finally, from field pH and temperature measurements, the DIC concentration can be recalculated to field $p\text{CO}_2$ in the water being analyzed. Potential advantages of the AHS method are: (1) simple and fast sample procedure in field, which is especially useful in certain environmental situations, such as when the air temperature is below freezing, and (2) an automated determination of CO_2 in the laboratory, which facilitates analysis of a large number of samples in a limited amount of time.

The study aims to:

- establish the accuracy of the AHS method for determining DIC;
- compare the AHS method to the more well-established DHS method for determining $p\text{CO}_2$; and
- evaluate the function of the AHS method in terms of practical and chemical limitations.

Methods

Detection of DIC with the AHS method

The accuracy of the DIC determination from the AHS method was tested against standard solutions of NaCO_3 . Solutions with different concentrations of DIC were prepared by diluting a stock solution of 76 200 μM NaCO_3 with distilled and ion-free water (Table 1).

Comparison of the AHS and DHS method

The AHS method was compared with a DHS technique used as a reference method because it is well established in the literature (Hesslein et al. 1991, Cole et al. 1994, Kelly et al. 2001, Jonsson et al. 2003). Similarities and dissimilarities were studied in both laboratory and field settings. The analyses in the laboratory were performed with sample water characterized by different combinations of 3 selected factors: DIC, pH, and water temperature (Tw). The 3 factors were varied in accordance with an inscribed central composite experimental design (CCD) in which each variable is varied independently of each other

(Table 1; Eriksson et al. 2000). To vary water temperature, the different water containers were either stored in an ice bath, a refrigerator, or at room temperature. Sample pH was varied by the addition of concentrated hydrochloric acid (HCl), in accordance with known pH-titration curves. Before sampling and analysis, the pH was allowed to stabilize during continuous stirring of the container.

To compare the 2 methods for measuring $p\text{CO}_2$ under field conditions, we applied both methods on one sampling occasion on the Krycklan/Degerö catchments (Buffam et al. 2007, Nilsson et al. 2008, Wallin et al. 2010, Laudon et al. 2011), where 14 stream sites were sampled on one day in December 2007. Any differences in lab or field $p\text{CO}_2$ between the AHS and DHS methods were checked using the nonparametric Wilcoxon signed-rank test.

Storage of samples

Although the vials used in the AHS method were prepared with HCl prior to sampling, which lowers pH of the sampled water to ~ 2 , any effects of respiration over time within the sample were checked in a storage test. Three stream sites (C2, C4, and C15) were sampled within the

Table 1. The experimental conditions, the measured DIC from the AHS method, and $p\text{CO}_2$ from the AHS and the DHS methods, respectively, and the detection of DIC and CO_2 , in relation to the standard DIC solutions and CO_2 determined with the DHS method, respectively.

Sample	Experimental conditions			AHS: Mean (n = 3)			DHS: Mean (n = 3)		Detection ^a	
	DIC μM	pH	Temp $^{\circ}\text{C}$	DIC μM	$p\text{CO}_2$ μatm	SD μatm	$p\text{CO}_2$ μatm	SD μatm	DIC %	CO_2 %
1	807	4.51	22.4	778	21 075	78	18 994	459	96	111
2	807	4.50	10.7	553	10 460	130	12 635	79	69	83
3	807	7.51	11.8	828	1476	12	1669	36	103	88
4	807	7.50	22.1	711	1516	68	1554	228	88	98
5	243	4.47	22.0	209	5590	1263	5525	557	86	101
6	525	5.99	16.7	342	5945	145	5042	356	65	118
7	243	7.49	10.2	247	450	25	587	39	102	77
8	243	4.51	9.2	236	4230	132	4317	408	97	98
9	243	7.51	21.9	246	513	16	661	60	101	78
10	525	6.01	14.5	520	8441	84	8419	402	99	100
11	50	5.99	18.0	57	1027	20	979	34	114	105
12	525	6.01	28.2	505	11 481	271	10 607	223	96	108
13	525	8.53	15.9	545	105	2	244	55	104	43
14	525	3.52	15.9	474	10 693	150	10 699	197	90	100
15	1000	6.01	16.6	1018	17 434	662	16 010	322	102	109
16	525	5.99	5.8	538	6838	106	6806	302	102	100
17	525	6.01	15.5	537	8901	141	8497	107	102	105

^aDIC detection: the amount of detected DIC in relation to the standard solutions of DIC. CO_2 detection: the amount of calculated CO_2 by the AHS method in relation to the DHS method.

Krycklan catchment on one day in March 2008 with 5 replicates taken per site. After sampling, 1 of the 5 samples from each site was immediately analyzed for DIC. The remaining samples were analyzed for DIC after 1, 3, 7, and 12 months. The samples were stored in the dark at 8 °C until analysis.

Sampling and analysis

Acidified headspace method (AHS)

For DIC determination (and subsequent $p\text{CO}_2$ calculation) a 5 mL sample of bubble-free water was injected into a 22 mL glass vial sealed with a rubber septa. The injection was made using a 10 mL syringe flushed with stream water (field study) or standard solutions (laboratory study) before sampling. The vial was pre-filled with 0.5 mL of 0.6% HCl and N_2 at atmospheric pressure. Samples were stored in the dark at 8 °C until analysis.

Direct headspace method (DHS)

For $p\text{CO}_2$ determination, a 545 mL glass bottle was filled with stream water (field study) or standard solution (laboratory study). The bottle was closed with a rubber cap below the water surface to keep the sample air-free. The rubber cap had 2, two-way valve connections to allow 2 syringes to be connected simultaneously. A headspace gas (50 mL) with a known molar fraction (ppmv) of CO_2 was added to the water-filled glass bottle with a plastic syringe, syringe 1 (S1). The excess volume of water (50 mL) was automatically transferred from the original water sample to syringe 2 (S2), thus maintaining atmospheric pressure within the bottle. The gas–water mixture was shaken for 1 min, and the headspace gas was extracted by injecting the excess water from S2 back into the glass bottle. This simultaneously transferred the headspace gas into S1, where it was isolated. Then 5 mL of the headspace gas was immediately transferred to evacuated and N_2 -filled vials for GC analysis. This dilution was accounted for in the calculations. During the laboratory study the temperature of the water was measured simultaneously with the gas analysis, and the air pressure in the lab was continuously logged with a calibrated portable barometer (Silva Alba Windwatch). During the field study, the headspace creation and isolation were conducted in a room at 10 °C because air temperatures were well below freezing (below –20 °C) at the time of sampling.

Headspace CO_2 concentrations were analyzed for both methods with a GC-FID (Perkin Elmer Autosystem Gas Chromatograph) equipped with a methanizer operating at 375 °C and connected to an auto sampler (HS40). Separation was carried out on a Haysep Q column using N_2 (40 mL min^{-1}) as a carrier gas (Nilsson et al. 2008,

Öquist et al. 2009, Wallin et al. 2010). The pH was measured for both the laboratory and the field study using an Orion 9272 pH meter equipped with a Ross 8102 low-conductivity combination electrode, which was calibrated before each measurement. Measurement of pH was done by gentle stirring of the sampled water. The effect of degassing of CO_2 while conducting the pH determination has been quantified by Buffam et al (2007) for the same instrument and sample treatment as used in this study. They concluded that for samples with $\text{pH} > 4.8$, this method resulted in slightly higher pH compared to closed-cell (field) pH. The precision of the pH determination was within 0.1 pH units of closed-cell pH.

Calculations

Acidified headspace method (AHS)

Because the samples were acidified ($\text{pH} \sim 2$), essentially all DIC was in the form of CO_2 , partitioned between H_2CO_3^* (where the asterisk indicates dissolved + hydrated CO_2) in the dissolved phase and CO_2 in the headspace. The $p\text{CO}_2$ in the equilibrated headspace ($p\text{CO}_{2\text{HS}}$) was determined using equation 2 (Coyne and Kelley 1974):

$$p\text{CO}_2 = X_{\text{CO}_2} \times P_{\text{air}}, \quad (2)$$

where X is the mole fraction of CO_2 (in ppmv). In applications that require high precision, the $p\text{CO}_2$ should also be corrected for non-ideal behavior and expressed as $f\text{CO}_2$ (Weiss 1974, McGillis and Wanninkhof 2006). The fugacity correction was not applied in this study, however, due to its low importance in relation to other errors (compare with Weiss 1974) and because it did not affect the comparison between methods. Hence, $p\text{CO}_2$ was recalculated to moles of CO_2 ($\text{CO}_{2\text{HS}}$) by using the ideal gas law (equation 3):

$$n_{\text{CO}_{2\text{HS}}} = \frac{p\text{CO}_{2\text{HS}} \times V_{\text{HS}}}{R \times T}, \quad (3)$$

where $n_{\text{CO}_{2\text{HS}}}$ is the amount of CO_2 in headspace in moles, $p\text{CO}_{2\text{HS}}$ is the partial pressure of CO_2 in the headspace (atm; equation 2), V_{HS} is the volume of headspace (L), R is the general gas constant (0.0820578; L atm mol^{-1} K $^{-1}$), and T is the temperature (K).

The concentration of DIC was calculated as a sum of the 3 fractions in the bicarbonate system: hydrated + dissolved CO_2 (H_2CO_3^*), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}):

$$[\text{DIC}]_{\text{water}} = [\text{H}_2\text{CO}_3^*]_{\text{water}} + [\text{HCO}_3^-]_{\text{water}} + [\text{CO}_3^{2-}]_{\text{water}}. \quad (4)$$

The concentration of each fraction was calculated according to equations 4, 7, and 8 (Stumm and Morgan

1996); equation 5 is Henry's law with Henry's constant (K_H) for CO_2 corrected for temperature according to equation 6:

$$[\text{H}_2\text{CO}_3^*] = \frac{p\text{CO}_{2\text{HS}}}{K_H}, \quad (5)$$

$$\ln K_H = A_1 + A_2 \frac{100}{T} + A_3 \ln \frac{T}{100}, \quad (6)$$

where A_1 , A_2 , and A_3 represent the constants -58.0931 , 90.5069 , and 22.2940 respectively (Weiss 1974); and

$$[\text{HCO}_3^-] = \frac{[\text{H}_2\text{CO}_3^*]K_1}{[\text{H}^+]}, \quad (7)$$

$$[\text{CO}_3^{2-}] = \frac{[\text{HCO}_3^-]K_2}{[\text{H}^+]}, \quad (8)$$

where $[\text{H}^+]$ is the proton concentration at pH 2, and K_1 and K_2 are the equilibrium constants in equations 9 and 10 according to Gelbrecht et al. (1998) adjusted for laboratory temperature:

$$\log K_1 = \left(\frac{-3404.71}{T} \right) + 14.844 - 0.033T, \quad (9)$$

$$\log K_2 = \left(\frac{-2902.39}{T} \right) + 6.498 - 0.0238T. \quad (10)$$

The total concentration of DIC in the sample was a simple sum of the headspace and the dissolved fraction:

$$[\text{DIC}]_{\text{Tot}} = \frac{n_{\text{CO}_{2\text{HS}}}}{V_{\text{Water}}} + [\text{DIC}]_{\text{Water}}, \quad (11)$$

where V_{Water} is the volume of sampled water.

To determine the CO_2 concentration in the stream during natural conditions, we used equation 12 (Stumm and Morgan 1996) together with in-stream pH and equilibrium constants (equations 9 and 10) adjusted for in-stream temperature. Because the dissolved CO_2 , (CO_2) (aq) is the dominant component of the H_2CO_3^* with a dissolved/hydrated ratio of 850:1 over a wide range of temperatures and ionic strengths (Soli and Byrne 2002), H_2CO_3^* was set equal to CO_2 (aq) and is referred to as CO_2 in the text:

$$[\text{CO}_2(\text{aq})] = \frac{[\text{DIC}]_{\text{Tot}}}{1 + \left(\frac{K_1}{[\text{H}^+]} \right) + \left(K_1 \frac{K_2}{[\text{H}^+]^2} \right)}. \quad (12)$$

Direct headspace method (DHS)

The $p\text{CO}_2$ of the headspace was calculated according to equation 2. The concentration of CO_2 in the water was calculated as follows:

$$[\text{CO}_2] = \frac{(n_{\text{CO}_{2\text{HS}}} + n_{\text{CO}_{2\text{w}}} + n_{\text{CO}_{2\text{HSstart}}})}{V_W}, \quad (13)$$

where $n_{\text{CO}_{2\text{HS}}}$ and $n_{\text{CO}_{2\text{w}}}$ are the respective amounts of CO_2 in the headspace and water, in moles, calculated according to equations 3 and 13, which are based on the ideal gas law and Henry's law. No fugacity correction was applied. The amount of CO_2 in the injected headspace before equilibration ($n_{\text{CO}_{2\text{HSstart}}}$) was calculated using equation 2 with the $p\text{CO}_2$ of the injected headspace gas:

$$n_{\text{CO}_{2\text{w}}} = K_H \times V_W \times p\text{CO}_2, \quad (14)$$

where K_H is calculated according to equation 6 (Weiss 1974).

Results

Accuracy and comparison with direct headspace method

The mean detection of dissolved NaCO_3 was 95% with the AHS method; however, in 2 samples only 65 and 69% of the expected DIC concentration were detected (Table 1). These low values were significantly lower than the others and also skewed the distribution of values around the mean. Because they probably were associated with error during preparation of the NaCO_3 solution, they were excluded and a new mean DIC detection value of 99% ($n = 15$) was calculated. With simulated randomized triplicate sampling from the data table of detection (without the 2 outliers), 5% of 10 000 simulated triplicate mean values were $<91\%$ or $>106\%$ (mean = 99%, with near normal distribution around the mean). Therefore, we expect a mean DIC detection accuracy of 99%, and that mean values based on triplicate sampling can be expected to be within 91–106% of the true DIC concentration in 95 of 100 cases.

The DIC detection was also independent of the concentration with similar detection across the full concentration range (Fig. 1). A regression model of the measured DIC concentration by the AHS method as a function of the standard concentrations had a slope of 1.0 and an intercept near the origin when the outliers were included. In the lab, no significant difference was observed between the AHS and the DHS methods in $p\text{CO}_2$ determination. The relative difference was, however, larger at low $p\text{CO}_2$ (Fig. 2). Above 1000 μatm , the detection of CO_2 ranged between 83 and 118% in relation to the DHS method (Table 1). Below 1000 μatm , the AHS method detected $<80\%$ in relation to the DHS method (Table 1).

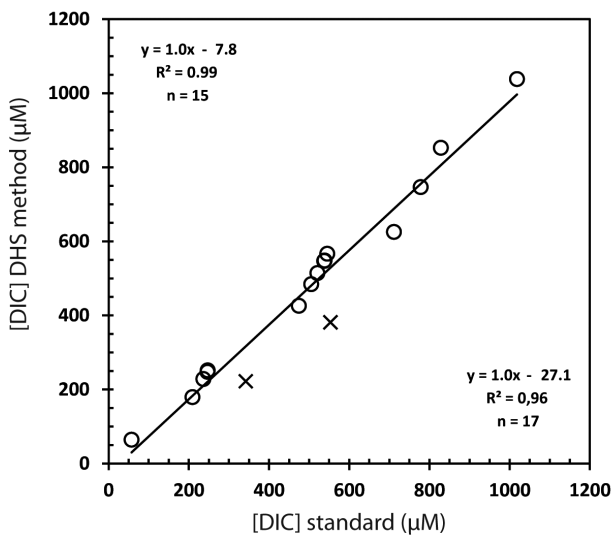


Fig. 1. DIC concentration determined by the direct headspace method (DHS; y-axis) as a function of the standard solutions of NaCO_3 (x-axis). The trend line is based on all 17 samples. Regression equations are given including all 17 samples and without the 2 outliers (crosses).

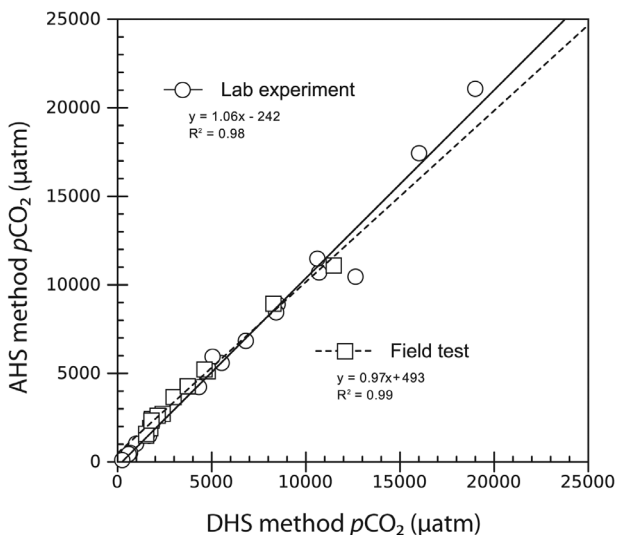


Fig. 2. Comparing results of $p\text{CO}_2$ determination between the direct headspace method (DHS; x-axis) and the acidified headspace method (AHS; y-axis).

The relative differences between the methods showed no significant correlations to the variation of DIC and T_w but was correlated to pH due to the low detection at $\text{pH} > 7$, where the smallest amounts of CO_2 were detected (Table 1, values < 1000 μatm). With the AHS method, variation among triplicates was generally lower (Table 1). The mean range (precision) between minimum and

maximum of the triplicates was $\pm 4.3\%$ for the AHS method and $\pm 6.7\%$ for the DHS method.

Field test

The AHS method showed significantly higher detection of CO_2 in the field in relation to the DHS method (mean difference = 370 μatm ; $p = 0.002$; Table 2), which is also shown by the intercept (493 μatm) in the regression model $p\text{CO}_2$ (AHS) as a function of $p\text{CO}_2$ (DHS; Fig. 2); however, the variation between replicates (precision) was similar to the lab tests (Table 2). In the field, the AHS method was generally simpler and faster than the DHS method. The vials are small and portable, and sampling could be accomplished in 1–2 and 4–5 minutes for the AHS and DHS methods, respectively (Table 3). The field sampling was performed at temperatures reaching below -20 $^\circ\text{C}$.

Storage test

During storage the DIC concentration increased in all 3 samples. The relative increase was 17–20% after 12 months, irrespective of DOC content and initial DIC concentration (Table 4). The initial speed of change showed large variability after 1 month and was faster at higher DOC. The mean increase of DIC (%) due to storage was well approximated with a logarithmic function: increase (%) = $0.054 + 0.057 \ln(x+0.38)$, $R^2 = 0.995$, where x = the number of months (Fig. 3). After 12 months the mean increase was 20%, while 1 month of storage of samples increased the headspace CO_2 concentration by 7%. According to the \ln -function, 3 days of storage would increase the DIC by 1.2%.

Discussion

Measurements of DIC and subsequent $p\text{CO}_2$ calculation with the AHS method as described in this study require little equipment and less time in the field compared to previous methods (Table 3). The DIC and CO_2 can also be determined from a single sample instead of 2 different samples, as with the DHS method. A major advantage of the AHS is therefore simpler operation that is approximately 3 times faster per sample during fieldwork.

Both DIC and CO_2 detection were tested in the study. The mean detection of DIC was nearly 100% with reasonable precision with triplicate sampling in the lab study (Table 1). For DIC measurements, the AHS may therefore be considered sufficient for many applications in freshwater systems. With respect to CO_2 detection, a major finding is that, in the lab, the AHS method yields similar results as the DHS method (Table 1). The field test

Table 2. $p\text{CO}_2$ values in stream water determined both with the DHS and the AHS methods. The sampling was performed in the Krycklan/Degerö catchments at outdoor temperatures below $-20\text{ }^\circ\text{C}$.

Site	pH	$p\text{CO}_2$ (n = 2)		CO ₂ detection % of DHS
		DHS μatm	AHS μatm	
C1	5.26	1511–1518	1475–1505	98
C3	3.90	8259–8293	8724–9150	108
C4	4.11	4763–4807	5091–5150	107
C6	5.05	1722–1741	1885–1930	110
C7	4.55	2350–2435	2668–2752	113
C10	4.82	1798–1831	2164–2683	134
C12	5.24	1482–1551	1560–1610	105
C13	5.49	3497–3934	4215–4323	115
C14	6.14	2119–2132	2567–2657	123
C16	6.32	1793–1799	2328–2336	130
C17	4.32	10 838–12 113	10 674–11 512	97
C18	4.32	4577–4650	5180–5263	113
C20	6.45	2955–2990	3638–3685	123
C22	5.47	3672–3775	4275–4287	115

Table 3. Scheme of the sampling procedures for the direct headspace method (DHS) and the acidified headspace method (AHS).

DHS	Time (s)	AHS	Time (s)
Set-up time	60	Set-up time	30
Sampling	60	Sampling	10
Headspace injection	30	Filling of vial	10
Equilibration	60	pH sampling	20
Headspace removal	30	T measurement	30
Filling of vial	10		
Temperature measurement	30		
Sum	280	Sum	100

showed that the AHS method produced significantly higher $p\text{CO}_2$ (mean 10%) than the DHS method. Because this discrepancy was not shown in the lab test, we believe it was caused by the extreme climate conditions (below $-20\text{ }^\circ\text{C}$), which complicated the sampling procedure and sample handling in field with the DHS method.

Calculations of CO_2 from pH have been questioned due to uncertainties in the measurements of field pH (Hope et al. 1995). It has even been suggested that pH in

slightly alkaline waters (pH just above 5.6.) could be more accurately determined by calculations from measurement of $p\text{CO}_2$ and DIC (Herczeg et al. 1985). Measurements of pH as done in this study (in the lab using a well-calibrated, low-conductivity combination electrode) have been shown to be within 0.1 pH units of closed-cell pH for a representative pH range (Buffam et al. 2007). A theoretical sensitivity analysis of measured pH on the $p\text{CO}_2$ calculation was conducted for the AHS method. A maximum error of +0.1 pH unit was used, resulting in an underestimation of the $p\text{CO}_2$ of <5% for samples with a pH <6.0 and of <12% for samples with a pH between 6.0 and 7.0.

The maximum potential error in $p\text{CO}_2$ for more alkaline samples (>7.0) is theoretically >12% with the given precision of the pH determination. The discrepancy in $p\text{CO}_2$ between the AHS and the DHS methods was generally >12% in the lab samples with a pH >7.0 (Table 1), but this discrepancy was likely more influenced by the uncertainty related to low $p\text{CO}_2$ samples and the sampling procedure of the DHS method used in this study rather than a pH effect (see further discussion in following paragraph); however, the accuracy and precision of the pH determination is important to the accuracy of the calculated $p\text{CO}_2$ using the AHS method. For high accuracy, we recommend the use of high quality combined pH–temperature electrodes (well-calibrated) that give a relatively quick pH reading.

Table 4. DOC and DIC concentrations (μM) measured from 3 stream sites in March 2008 within the Krycklan catchment. Replicates of DIC were analyzed after 1, 3, 7, and 12 months (% increase in DIC concentration is given in parenthesis).

Site	DOC	DIC (μM) after (n) months				
		0	1	3	7	12
C2	975	156	166 (+6%)	174 (+12%)	188 (+21%)	187 (+20%)
C4	2825	477	530 (+11%)	550 (+15%)	552 (+16%)	558 (+17%)
C15	842	138	142 (+2%)	153 (+10%)	159 (+15%)	166 (+20%)

With respect to CO_2 determination and calculation in the lab test, the 2 methods yielded similar results, especially at concentrations $>1000 \mu\text{atm}$ (Table 1). The lower CO_2 detection with the AHS method $<1000 \mu\text{atm}$, and especially at the lowest concentrations, can be explained by contamination of CO_2 in the evacuated vials because the correction for dilution becomes increasingly sensitive to CO_2 residues in the vial at low concentrations of the injected gas. A residual concentration of $30 \mu\text{atm}$ CO_2 in the evacuated vial would explain the difference observed in sample 13 (Table 1). The measured residual concentrations in the vials before sampling were 5–10 ppm ($n = 6$), and we therefore conclude that this, together with some air contamination during the sampling procedure, probably caused most of the difference between the methods at the lowest concentrations. The unavoidable, but still small, CO_2 contamination from the air due to sampling, together with the high costs involved in getting ultra-low CO_2 residues in the vials, also

highlights that the DHS method preferably should be used with portable infrared gas analyzers (IRGAs) or similar equipment for instant aqueous CO_2 measurement at sites with low CO_2 concentrations.

The potentially faster sampling rate with the AHS method is partly because the sample water is directly injected into a prepared storage container (the vial), and the headspace equilibrates before analysis without any additional shaking time. Because not only gas but also water is stored until analysis, it is essential to preserve the sample without any influence from degradation of organic carbon in the water. The storage test indicates that microbial degradation occurred despite the low pH (~ 2). Further, the rate of increase was logarithmic, and samples with high DOC may have the highest initial rate of increase.

Collectively, the nature of the observed increases indicates that analysis of the samples should be done within a few days to minimize effects of in-sample respiration, especially if the water has a high concentration of DOC. The DIC increase was $1\text{--}20 \text{ mg m}^{-3} \text{ d}^{-1} \text{ C}$ during the first 30 days (based on the data from Table 4), which was much slower than average bacterial respiration rates ($<200 \text{ mg m}^{-3} \text{ d}^{-1} \text{ C}$) measured in incubation experiments of the stream waters of the Krycklan catchment (Berggren et al. 2007, 2009). The rates were, however, similar to the lowest measured rates of planktonic respiration in lakes ($8 \text{ mg m}^{-3} \text{ d}^{-1} \text{ C}$) but much slower than average planktonic respiration rates in lakes ($188 \text{ mg m}^{-3} \text{ d}^{-1} \text{ C}$; Pace and Prairie 2005).

The comparison of the variation between replicates indicated that the AHS method had better precision in most cases (Table 1) because the headspace gas of the DHS method must be moved from the bottle to a storage container and, in this case, also was diluted (Table 3). A better precision and overall performance with the DHS method can thus be expected if the analysis of the headspace gas is done *in situ* with undiluted gas and a portable IRGA. If low concentrations are expected, we recommend optimizing the headspace volume to ensure that the GC operates well above its detection limit. Note that the highest concentration of CO_2 in the headspace can be achieved with an AHS method.

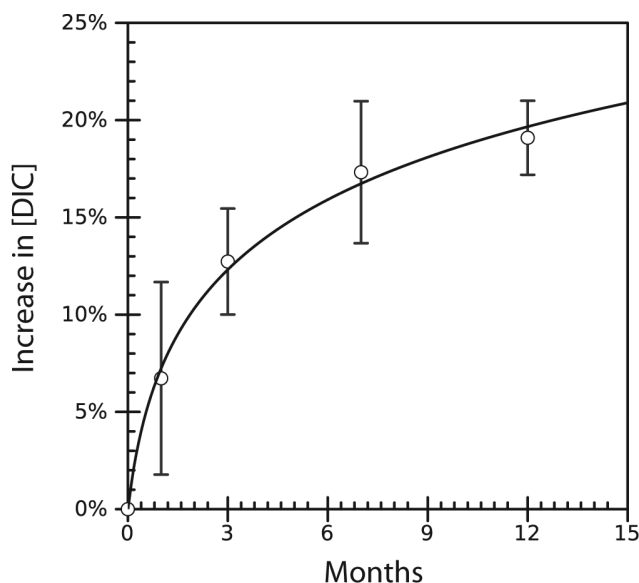


Fig. 3. Percentage increase in DIC concentration, [DIC], as a function of storage time (months) using the acidified headspace method (AHS). The samples were stored dark and cold ($8 \text{ }^\circ\text{C}$) prior to analysis. Average values of 3 different stream sites are presented, with error bars showing standard deviation.

Despite limited possibilities to optimize both methods, the study shows that neither the AHS method nor the DHS method is likely to yield high precision, especially during field conditions. The DIC-detection accuracy of the AHS method is, however, sufficient for many applications with the triplicate-based precision of approximately $\pm 5\%$ and with a linear detection over the full concentration range of this study (Fig. 1). With the DHS method, the low air temperature in the field caused expansion of the water in the sample during sample preparation, and a slight gas loss was difficult to avoid. The loss of headspace gas hence lowered the values of CO_2 , which explains the lower detection of CO_2 with the DHS method in the field (Fig. 2). Water freezing in the sampling bottles could be avoided with a portable IRGA, extra batteries (or other portable power sources), and a heated tent close to the sampling site (A. Jonsson, pers. comm.). In contrast, the great advantage of the AHS method is its simple operation in the field and the faster sampling rate compared to the DHS method. As long as analysis of the samples can be completed within a few days (we recommend 3 days) the AHS method is especially useful at sampling during severe weather conditions, such as extreme cold, or during time-limited sampling campaigns, such as synoptic surveys that cover large geographical areas with several samplers.

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