Comparative performance of CO2 measuring methods: Marine aquaculture recirculation system application

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ABSTRACT

Many methods are available for the measurement of dissolved carbon dioxide in an aqueous environment. Standard titration is the typical field method for measuring dissolved CO2 in aquaculture systems. However, titrimetric determination of dissolved CO2 in marine water aquaculture systems is unsuitable because of the high dissolved solids, silicates, and other dissolved minerals that interfere with the determination. Other methods used to measure dissolved carbon dioxide in an aquaculture water included use of a wetted CO2 probe analyzer, standard nomographic methods, and calculation by direct measurements of the water’s pH, temperature, and alkalinity. The determination of dissolved CO2 in saltwater based on partial pressure measurements and non-dispersive infra-red (NDIR) techniques with a CO2 gas analyzer are widely employed for oceanic surveys of surface ocean CO2 flux and are similar to the techniques employed with the head space unit (HSU) in this study. Dissolved carbon dioxide (DC) determination with the HSU using an infra-red gas analyzer (IRGA) was compared with titrimetric, nomographic, calculated, and probe measurements of CO2 in freshwater and in saltwater with a salinity ranging from 5.0 to 30 ppt, and a CO2 range from 8 to 50 mg/L. Differences in CO2 measurements between duplicate HSUs (0.1–0.2 mg/L) were not statistically different. The coefficient of variation for the HSU readings averaged 1.85% which was better than the CO2 probe (4.09%) and that for the titrimetric method (5.84%). In all low, medium and high salinity level trials HSU precision was good, averaging 3.39%. Differences existed between comparison testing of the CO2 probe and HSU measurements with the CO2 probe readings, on average, providing DC estimates that were higher than HSU estimates. Differences between HSU and titration based estimates of DC increased with salinity and reached a maximum at 32.2 ppt. These differences were statistically significant (P<0.05) at all salinity levels greater than 0.3 ppt. Results indicated reliable replicated results from the head space unit with varying salinity and dissolved carbon dioxide concentrations.

1. Introduction

In intensive recirculating aquaculture systems (RAS) metabolic-derived carbon dioxide can accumulate if there is a lack of degassing processes or limited system water exchange. Elevated dissolved CO2 concentration in the water will activate the primary stress responses in fish causing significant physiological disturbances that can result in reduced growth, poor feed conversion, nephrocalcinosis, or hypercalciumosis (Smart et al., 1979; Smart, 1981; Perry et al., 1989; Wedemeyer, 1996; Fivelstad et al., 1999). Dissolved carbon dioxide (DC) in an RAS is most often controlled through air stripping including use of the packed column, agitators, or diffused aeration and by the addition of base reagents such as sodium hydroxide or quick lime or by a combination of both methods (Bisogni and Timmons, 1994; Watten et al., 2004). Since carbon dioxide build-up in these systems can be a limiting factor to production and production efficiency the removal of CO2 should be an important design consideration as well as the ability to monitor the CO2 levels in the systems.

When carbon dioxide dissolves in water the reactions which take place can be represented by the following series of equilibria: (Stumm and Morgan, 1996)

\[ \text{CO}_2(g) = \text{CO}_2(aq), \]
\[ \text{CO}_2(aq) + \text{H}_2\text{O}(l) = \text{H}_2\text{CO}_3(aq), \]
\[ \text{H}_2\text{CO}_3(aq) = \text{H}^+(aq) + \text{HCO}_3^-(aq), \]
\[ \text{HCO}_3^-(aq) = \text{H}^+(aq) + \text{CO}_3^{2-}(aq), \]

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The notations (g), (l), (aq) refer to the state of the ion or molecule, i.e., a gas, a liquid, or in aqueous solution respectively. Usually, \( \text{CO}_2\text{aq} \) and \( \text{H}_2\text{CO}_3\text{aq} \) are grouped together and expressed as the sum concentration of \( \text{CO}_2\text{aq} \) because it is difficult to distinguish between the two ions. Basically, two of the parameters of the carbon dioxide system (total alkalinity and the total hydrogen ion concentration, \( \text{pH} \)) can be measured to make an estimate of the carbon dioxide in aqueous solution.

Numerous methods have been developed for measuring the parameters of the marine carbonate system to characterize the oceanic \( \text{CO}_2 \) fluxes. The methods and techniques developed are very complex compared to head space reactor design and dissolved \( \text{CO}_2 \) calculation methods for aquaculture purposes presented in this paper. Precise and accurate measurement of the marine carbonate system parameters are required in determining the effects of the anthropogenic \( \text{CO}_2 \) increases on the global carbon cycle and climate research effort. The various methods include \( \text{CO}_2 \) analyzers that use the principle of membrane diffusion thermal conductivity, potentiometric titration, total \( \text{CO}_2 \) by gas extraction (manometry, coulometry), or partial pressure measurements by gas equilibrium using gas chromatography or a non-dispersive infra-red gas analyzer system (Schumacher and Smucker, 1983; Copin-Montegut, 1985; Johnson et al., 1985; Bradshaw and Brewer, 1988; Wanninkhof and Thoning, 1993; Takahashi et al., 2002; Bandstra et al., 2006). Development of non-dispersive infra-red (NDIR) techniques have yielded total dissolved carbon dioxide measurements for flowing seawater stream with 0.1% accuracy and precision with a e-folding time (travel time through sampling tube) of 7–10s (Bandstra et al., 2006). The highly precise and accurate NDIR techniques employed for oceanic surveys of surface ocean total dissolved \( \text{CO}_2 \) flux are similar to the techniques we used to determine the \( \text{pCO}_2 \) presented in this paper with the head space unit and are applicable for dissolved \( \text{CO}_2 \) determination in saltwater recirculating aquaculture systems.

Typical \( \text{pCO}_2 \) systems that employ NDIR techniques are comprised of sampling hardware, an analysis system, and a data logging system. The analysis system includes an equilibrator (i.e., head space unit), ancillary data collection equipment to measure temperature and pressure, a sampling seawater supply and drain line, condenser to remove moisture from the sample gases, and a \( \text{CO}_2 \) infra-red gas analyzer, IRGA (i.e., the handheld CEA GD 444 instrument). Similar to the head space unit (HSU) used in this study an equilibrator ensures that the partial pressures of \( \text{CO}_2 \) in the water sample and head space gases are in equilibrium. Other challenges encountered by scientists operating \( \text{pCO}_2 \) systems for ocean surface \( \text{pCO}_2 \) studies include temperature difference between water intake and equilibrator and pressure changes in the equilibrator resulting from unstable water flows. Such issues should not be encountered with the head space unit because it is implemented in close proximity to the system where DC is being measured (<3 m) and temperature or pressure differences are nonexistent between the source water and head space unit. In situations where high precision and accuracy of the \( \text{pCO}_2 \) is required these effects can significantly effect the resulting \( \text{pCO}_2 \) measurement from the infra-red gas analyzer, IRGA (Li-Cor, Inc. Application Note 116 and 117). Recent improvements in both infra-red spectroscopy and equilibrator techniques have allowed \( \text{pCO}_2 \) determination in highly turbid and over saturated \( \text{CO}_2 \) waters (Frankingnoulle and Borges, 2001) thus making the gas streaming infra-red technique for \( \text{CO}_2 \) determination very applicable to aquaculture applications.

In commercial and general aquaculture research applications the need for such accuracy and precision is limited and the associated cost for the gas-streaming infra-red techniques is potentially prohibitive. In aquaculture applications the \( \text{pH} \)/alkalinity and the titrimetric Standard 4500–\( \text{CO}_2 \) methods by American Public Health Association (APHA, 1998) are often the most utilized and cited. The shortcomings of these APHA standard methods is that the titration of \( \text{CO}_2 \) is not recommended for waters of high total dissolved solids, such as those encountered in seawater and the \( \text{pH} \)/alkalinity method is subject to the limitations of accurate \( \text{pH} \) measurement. Glass electrode \( \text{pH} \) assemblies have shown to produce misleading \( \text{pH} \) measurements in samples of high ionic concentration, such as seawater (Ilingworth, 1981; Covington and Whitfield, 1988; Marcus, 1989). Other instrumentation suitable for measuring carbon dioxide in an aquaculture environment is the recently developed gas-permeable membrane portable \( \text{CO}_2 \) meter manufactured by OxyGuard International (Bikerød, Denmark). The portable \( \text{CO}_2 \) meter utilizes a gas-permeable membrane and infra-red adsorption cell to directly measure the \( \text{pCO}_2 \) in aqueous solutions. A built-in thermistor accounts for the temperature effect on solubility and the effect of salinity is accounted for during the calibration procedures. At the time of the study this was the only meter commercially available to quantify \( \text{CO}_2\text{aq} \). However, gas-permeable membranes separating probe electrolytes from water can generally be susceptible to biological fouling (Speirs et al., 1986; Watten et al., 1997).

Watten et al. (2004) evaluated the HSU for \( \text{CO}_2 \) monitoring in freshwater aquaculture systems which circumstances problems associated with wetted gas-permeable membranes. Carbon dioxide concentration is calculated using Henry’s Law, water temperature, and the partial pressure of carbon dioxide (\( \text{pCO}_2 \)) that develops in the head space of a vertical gas–liquid contacting chamber. Gas-phase partial pressures were determined with either an IRGA or by measuring voltage generated by a \( \text{pH} \) electrode immersed in an isolated sodium carbonate solution (SC) sparged with recirculated head space gas. Calculated DC concentrations were compared with those obtained by titration over a range of DC (2–28 mg/L), total alkalinity (35–250 mg/L as \( \text{CaCO}_3 \)), total dissolved gas pressure (–178 to 120 mm Hg), and dissolved oxygen concentrations (7–28 mg/L). Statistically significant correlations were established between head space IRGA measurements and titrimetrically determined DC concentrations. Millivolt and titrimetric values from the SC solution tests were also correlated and the absolute and relative errors associated with the use of the IRGA and SC solution averaged 0.9 mg/L DC and 7.0% and 0.6 mg/L DC and 9.6%, respectively. The precision of DC estimates determined by the coefficient of variation for the head space IRGA, SC solution, and titration analyses were low, averaging 1.0%, 1.3% and 1.7%, respectively.

In this study, the HSU with an IRGA described by Watten et al. (2004) was evaluated for saltwater recirculating system application and its performance was compared to alternative methods of DC measurement. The alternative methods included standard methods of titration, nomographic, and calculation (APHA, 1998), use of the portable \( \text{CO}_2 \) meter by OxyGuard, and an indirect method using a \( \text{pH} \) and \( \text{pCO}_2 \) correlation curve.

2. Materials and methods

2.1. Instrument description

The head space unit is designed to promote equilibrium between dissolved gas tensions (\( P_i^G \)) and gas phase partial pressures (\( P_i^L \)) within a gas–liquid contacting chamber, Fig. 1. Thus, for individual gas species:

\[
P_i^L = P_i^G
\]
of an individual gas species partial pressure, chamber, respectively. This action would result in some deviation
conditions will cause gas movement into and out of the head space
and BP is atmospheric pressure in mm Hg.

\[ \Delta_1 P = \Delta G - \Delta H \]

and for all gases present:

\[ \left( \sum_{i}^{n} P_{i}^{G} \right) + P_{H_2O} = \left( \sum_{i}^{n} P_{i}^{L} \right) + P_{H_2O} \]

where \( P_{H_2O} \) represents water vapor pressure (Colt, 1984), and all \( P \) values are expressed in mm Hg. From Dalton’s Law, the sum of terms on the right side of Eq. (2) represents total gas phase pressure (absolute). The sum on the left side represents total dissolved gas pressure (TDGP). The head space chamber is vented to the atmosphere to prevent internal water level fluctuations that may result from gas absorption or release by water directed through the instrument. Hence, the equalities given in Eqs. (1) and (2) will be true only when TDGP is equal to atmospheric pressure (BP), i.e., \( \Delta P = 0 \) where

\[ \Delta P = \text{TDGP} - \text{BP} \]

and BP is atmospheric pressure in mm Hg.

It follows that hypobaric (\( \Delta P < 0 \)) and hyperbaric (\( \Delta P > 0 \)) conditions will cause gas movement into and out of the head space chamber, respectively. This action would result in some deviation of an individual gas species partial pressure, \( P_{i}^{G} \), from their corresponding gas tension, \( P_{i}^{L} \). The instrument design shown in Fig. 1 provides for the measurement of head space partial pressure (\( P_{i}^{G}, \text{CO}_2 \)) using an infra-red gas analyzer that incorporates an internal gas-sampling pump (Model GD-888, CEA Instruments, Inc, Westwood, NJ). Water continuously flows into the head space unit during the gas sampling procedure. The volume of the HSU reservoir chamber is 55 L but the five 1.8 cm diameter drain holes in the chamber set the reservoir water volume at approximately 37 L. The flow through the head space reactor was 10–11 L min\(^{-1}\), considered a high flow rate for typical oceanic pCO\(_2\) monitoring systems. The higher flow rate minimizes any potential error resulting from accumulation and subsequent decomposition of organic matter and is set by the water height in the HSU head box, and the number and diameter of holes in the distribution plate. Sample pumping to the head space unit is not required as the head requirements have been considered in the design to take advantage of the elevation difference that exists between production system components and eliminates those pumping risks and costs (Watten et al., 2004). An additional consideration was a water vapor barrier in the gas sample tubing to reduce the moisture content of the gas sample. A 0.2–0.5 μm PTFE filter was utilized to dry the influent gas sample as well as prevent water vapor damage to the analyzer. It is important to note that the water vapor in the gas stream absorbs infra-red in a similar part of the spectrum as CO\(_2\) and should be eliminated as much as reasonable possible. The IRGA used to collect the gas sample from the HSU has a built-in pump with push button control that operates for 15 s when pushed to obtain the 50 mL gas sample. Usually two continuous samples can be drawn before additional time is needed to equilibrate additional head space gas for sampling. The response time is typically 20 s and the accuracy of the IRGA unit is ±5% of the reading. The auxiliaries of the IRGA instrument includes data logging and recording output, relay, and alarming capabilities. The CO\(_2\) range for the standard IRGA model from CEA Instruments is 0–1% however, the portable handheld unit used in the trials had the extended CO\(_2\) range, 0–10% with a list price of $2,495.00. The calibration kit was ordered ($425.00) with the instrument to perform routine calibration prior to use. The kit contains a cylinder of CO\(_2\) gas of known percent, a special preset regulator and tubing. During calibration procedures a length of tubing (approximately 3 m in length and 1 cm inside diameter) was infused with CO\(_2\) from the calibration cylinder so the IRGA draws a sample under ambient static conditions rather than under pressure. The manufacturer recommends annual instrument factory calibration of the IRGA at a cost of approximately $550.00.

This infra-red gas analyzer provides a percent by volume CO\(_2\) measurement (wet basis) from which \( p_{i}^{G, \text{CO}_2} \) is calculated:

\[ p_{i}^{G, \text{CO}_2} = \text{BP} \left( \frac{\%\text{CO}_2}{100} \right) \]

\( p_{i}^{G, \text{CO}_2} \) values estimate carbon dioxide tension (\( p_{i}^{G, \text{CO}_2} \), Eq. (1)), from which DC can be established (mg/L) using the Bunsen solubility coefficient (\( \beta \)) determined as a function of water temperature and salinity (Colt, 1984):

\[ \text{DC} = \beta_{\text{CO}_2} \left( \frac{p_{i}^{G, \text{CO}_2}}{0.3845} \right) \]

where \( \beta_{\text{CO}_2} \) is in units of L gas/L water-atmosphere, and \( p_{i}^{L, \text{CO}_2} \) is in units of mm Hg. The factor 0.3845 equals 760/(1000k) and \( k \) represents the ratio of molecular weight to molecular volume for carbon dioxide gas (Colt, 1984). The head space unit used in this study (Fig. 1) differs from that described by Watten et al. (2004). The original cone diffuser designed to minimize bubble carryover was replaced with a standard 5.1 cm × 10.2 cm expansion coupling and the gas sample ports were repositioned so as to eliminate interaction between gas inlet and outlet streams.

2.2. CO\(_2\) methods of measurement

The readings from the head space unit using the IRGA were compared, when possible, to alternative methods of DC measurement including titration, DC membrane probe, calculation using pH and
alkalinity, and the nomograph method. A brief description and the test series the method was used for is provided below.

1. Nomographic determination of free carbon dioxide, Standard Method 4500-CO₂ B. This method is based upon equations relating the ionization equilibria of the carbonates and water. The water temperature, total dissolved solids content, pH, and total alkalinity are needed to determine the free CO₂ concentration. Alkalinity is measured using HACH method 82030. This method is not recommended for waters of high total dissolved solids, such as those encountered in seawater. Method used in Test Series 3.

2. Titrimetric method for free carbon dioxide, Standard Method 4500-CO₂ C. Free CO₂ reacts with sodium carbonate or sodium hydroxide to form sodium bicarbonate. Completion of this reaction is indicated by the development of a pink color at the equivalence pH of 8.3 using a 0.001 N sodium bicarbonate indicator solution. Method used in Test Series 1 and 4.

3. Carbon dioxide determination by calculation, Standard Method 4500-CO₂ D. The calculation is subject to the same ionization equilibria of the carbonates as the nomographic procedure and the additional restriction of using a single temperature of 25 °C. Method was used in Test Series 3.

4. A portable CO₂ meter (Oxyguard® CO₂ Portable Analyzer, Oxyguard International, Birkerod, Denmark). The Oxyguard® CO₂ portable meter measures the free dissolved CO₂ in the systems and is not affected by carbonates or other dissolved substances. The probe was used in all the Test Series except Series 4.

5. An indirect method as described by Watten et al. (2004) that estimates pCO₂ given the pH of the test water representing a specific temperature and salinity, and a regression equation correlating pLCO₂ versus pH for the same water condition. The apparatus used to correlate pLCO₂ with pH is shown in Fig. 2. This method was used in Test Series 3.

2.3. Test Series 1—CO₂ measurement in freshwater

The head space unit was used to measure DC in spring water that was approximately 15.5 °C, had a total dissolved solids concentra-
tion of 0.6 g/L, and was sparged with carbon dioxide to establish DC concentrations of approximately 20, 40, and 50 mg/L, which is within the \( \text{CO}_2_{[aq]} \) measurement range of the OxgyGuard CO2 meter. Results were compared to measurements obtained by titration (Method 4500-CO2C, APHA, 1998) and by the Oxyguard \(^{\text{R}}\) CO2 Portable Meter (referred to as the CO2 probe from here on out). Fig. 3 provides a plan view of the experimental setup that included a 2.3 m\(^3\) capacity water reservoir, diffusers used to sparge commercial-grade compressed \( \text{CO}_2 \) into test water when required, a 0.1 kW centrifugal pump to deliver reservoir sample water to each of the two head space units, and a sample port for collecting water samples for alkalinity determination (APHA, 1998). Duplicate probes for measuring pH (Model DPD1P1 pH sensors; Model sc100 Standard Controller, HACH Co., Loveland, CO) were suspended in the reservoir as well as a probe for measuring the dissolved oxygen concentration and salinity (Model Y-85, YSI Inc., Yellow Springs, OH). The pH probes are general purpose glass electrodes that use the differential electrode technique for pH measurement with a sensitivity of ±0.005 pH and an operating temperature range of −5 to 70°C. The pH probes were calibrated daily and before each Test Series using standard 4, 7, and 10 NIST (National Institute of Standards and Technology) buffer solutions. The calibration procedure used to calibrate the CO2 probe was based on the recommended procedures supplied by the manufacturer and was calibrated at each salinity concentration.

In operation, the test reservoir was filled with spring water, and sparged with commercial grade compressed \( \text{CO}_2 \) to obtain the approximate DC concentrations stated above. The HSU was used to determine the \( \text{CO}_2 \) in the reservoir during the sparging procedure. When the desired DC concentration was reached, flow from the reservoir was directed through replicate HSU's at a flow rate of approximately 10 L min\(^{-1}\) per unit. Percent \( \text{CO}_2 \) readings from the unit’s head space were recorded when readings had stabilized. Concurrently, water samples were taken for DC measurement by titration and analyzed immediately. Dissolved oxygen concentration, barometric pressure, and reservoir water temperature were also recorded. Local barometric pressure was measured with a pressure transducer (Model 2019 Modumeter, Solomat Inc., Stamford, CT). DC estimates provided by the HSU instruments were calculated based on Eqs. (4) and (5). Sampling by the HSU, titration, and CO2 probe were repeated three times at approximately thirty minute intervals.

2.4. Test Series 2—CO2 measurement at varying salinity levels

The tests described above for the HSU and CO2 probe were repeated at four salinity levels: 4.5, 8.2, 15.6, and 29.8 ppt. Salinity of the reservoir water was increased to desired concentrations by addition of pre-determined amounts of a commercial sea salt blend (Instant Ocean®, Aquarium Systems Inc., Mentor, OH) and the DC was elevated to a concentration between 40 and 50 mg/L using the same sparging procedure stated above in Section 2.3 with temperature held at approximately 17.5°C. Salinity levels were measured with an YSI Model Y30 SCT probe (Yellow Springs Instruments, Inc., Yellow Springs, OH). DC was not measured by titration due to the high levels of total dissolved solids (APHA, 1998).

2.5. Test Series 3—CO2 measurement at varying salinity and DC levels

In the third set of tests, DC versus salinity was evaluated in freshwater, and two different salinities (15.2 and 30 ppt) using all five methods of measurement. Readings were repeated three times for a single DC concentration and salinity combination to obtain a mean DC value for each of the five test methods. Measurements of pH were obtained from each of the pH probes suspended directly in the test solution reservoir (Fig. 3) and used independently to determine the \( \text{CO}_2 \) concentration in the test waters. CO2 measurements were repeated three times for each DC and salinity level combination to obtain a mean DC value for each of the test methods.

When using the indirect method, Method 5, the vented one liter equilibrator bottle in the schematic (Fig. 2) was filled with 400 mL of water. A mixture of air and commercial grade compressed \( \text{CO}_2 \) was then directed through the sparging system and the apparatus outfitted with a pH and temperature probe (Accumet® Model AR50, Thermo Fisher Scientific, Inc., Pittsburgh, PA). Gas flow was fixed at 10.5 L min\(^{-1}\). The sparging action promotes movement of \( \text{CO}_2 \) in or out of the test solution so that the equilibrium between liquid and gas phase \( \text{CO}_2 \) can be established. Induced changes in \( \text{pH} \) affects the hydrogen ion concentration in the test sample following the reaction:

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{HCO}_3^- \leftrightarrow \text{H}^+ + \text{CO}_3^{2-}
\]  

(6)

The extent of the change in \( \text{H}^+ \) is related to temperature and alkalinity. A water bath was used to match test sample temperature with bulk reservoir temperature. The response of the pH probe to gas mixtures representing 0.2–4.5% \( \text{CO}_2 \) on a volumetric basis as determined by the IRGA was measured. Measured % \( \text{CO}_2 \) values were used to calculate a corresponding \( \text{pH} \), given the local barometric pressure, BP (Eq. (4)). Results were then modeled with regression to provide the correlations needed to convert pH measurements in the reservoir with \( \text{pH}_{50} \), assuming calculated \( \text{pH}_{50} \) the calculated value of \( \text{pH}_{50} \) is obtained from the appropriate regression equation of the modeling results graphed in Fig. 4. The resulting \( \text{pH}_{50} \) is then used to calculate DC as presented in Eq. (5).

Statistical analyses for evaluating difference in the mean values amongst the treatment groups were conducted using either the Statistical Analysis System (SAS, Version 8.0) or Sigma Stat (Version 2.0.3) software. Specific analyses included linear regression, one-way analysis of variance, and Tukey’s Test for multiple pairwise comparisons of treatment means.

2.6. Test Series 4—CO2 measurements at elevated salinity levels with the HSU and titration

This set of tests compared HSU DC measurements and DC determinations by titrimetric methods at elevated salinity concentrations to quantify errors in titration related to elevated total dissolved solids as predicted by APHA (1998). This set of tests was conducted at the USDA – ARS Sustainable Marine Aquacul-
ture Systems facility at the Harbor Branch Oceanographic Institute, Florida Atlantic University Aquaculture Park in Fort Pierce, FL. Test water was a blend consisting of the location’s saltwater well source (32.7 ppt) and a freshwater source. Freshwater and salinities of 5.0, 10.7, 15.5, 20.5, 25.0, and 32.7 ppt were tested. CO2 was added to a 1.0 m³ reservoir tank (0.76 m diameter × 2.1 m height) by sparging commercial grade compressed CO2 gas through a medium pore air diffuser into the water filled reservoir. Water in the reservoir was gently continuously stirred to minimize degassing of the CO2 in the closed reservoir with a 35-watt Mag Drive pump (Aquatic Eco-Systems, Model no. MD3, Apopka, FL). Specific analyses of the sample water in Test Series I for evaluating the head space units and CO2 probe and the CO2 membrane probe or the titration method. As shown in Table 1, at the low CO2 concentration there was no difference between the head space units and the CO2 probe. The CO2 concentration determined using HSU 1 and 2 were 21.5 ± 0.8 and 21.6 ± 0.2 mg/L and the CO2 probe was 22.7 ± 0.8 mg/L (mean ± SD). Titration did yield a much higher result, 28.1 ± 4.3 mg/L which was only significantly different from HSU 1. At the medium and high concentrations the CO2 probe results were significantly different than the result from the HSU 1 and 2 and titrimetric results. At the medium CO2 concentration, HSU 1 and 2 yielded 38.3 ± 0.4 and 38.2 ± 0.5 mg/L, respectively. The CO2 probe yielded 42.3 ± 0.9 and titration results were 40.1 ± 0.6, both significantly different than the HSU results (P < 0.05) and each other. At the high CO2 concentration trial, HSU 1 and 2 and the titration results for CO2 (51.5 ± 1.0, 50.7 ± 1.0, and 55.2 ± 0.4 mg/L) were significantly different (P < 0.05) from the CO2 probe measurements (61.9 ± 4.1 mg/L).

3.2. Test Series 2

Test Series 2 results are summarized in Table 2. BP ranged from 750.0 to 751.3 mm Hg, and the water temperature decreased during the trial the dissolved oxygen (DO) concentration increased. The temperature decreased from 18.8 °C in the high salinity trial (29.8 ppt) to 16.6 °C for the low salinity trial (4.5 ppt) as the reservoir water was diluted with the cool spring water. The DO correspondingly increased from 8.3 to 10.1 mg/L, respectively. DC also dropped during the salinity dilution steps by the addition of the cool spring water. DC ranged from approximately 50 mg/L at the start of the trials dropping to roughly 40 mg/L CO2 with the spring water dilution. Differences between DC output of each HSU were minimal ranging from 0.2 to 1.0 mg/L and precision, as indicated by CV (2.08%) was comparable to the CO2 membrane probe (2.24%). The CO2 probe DC readings were statistically greater than the HSU for.

Table 1

<table>
<thead>
<tr>
<th>Barometric pressure, and dissolved oxygen concentration and temperature of the sample water in Test Series I for evaluating the head space units, CO2 probe, and the titrimetric method for determining CO2 (mg/L) in the range of 40–55 mg/L at four different salinity concentrations ranging from 4.5 to 30 ppt. Data in the same column with different letters differ significantly (P &lt; 0.05).</th>
<th>Low CO2</th>
<th>Ambient CO2</th>
<th>High CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head space unit 1</td>
<td>21.5 ± 0.8a</td>
<td>38.3 ± 0.4a</td>
<td>51.5 ± 1.1a</td>
</tr>
<tr>
<td>Head space unit 2</td>
<td>21.6 ± 0.2a,b</td>
<td>38.2 ± 0.5a,b</td>
<td>50.7 ± 1.0a</td>
</tr>
<tr>
<td>CO2 probe analyzer</td>
<td>22.7 ± 0.8a,b</td>
<td>42.3 ± 0.9a,b</td>
<td>61.9 ± 4.1b</td>
</tr>
<tr>
<td>Titrimetric method</td>
<td>28.1 ± 4.3b</td>
<td>40.1 ± 0.6b</td>
<td>55.2 ± 0.4a</td>
</tr>
<tr>
<td>Barometric pressure (mm Hg)</td>
<td>749.0</td>
<td>749.0</td>
<td>749.0</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>15.5</td>
<td>15.6</td>
<td>15.2</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>10.7</td>
<td>10.1</td>
<td>10.3</td>
</tr>
</tbody>
</table>

Each data point represents the means ± the standard deviation of three replicates. ND = no data was collected as titrimetric endpoint was unattained.

Table 2

<table>
<thead>
<tr>
<th>Salinity (ppt)</th>
<th>4.5</th>
<th>8.2</th>
<th>15.6</th>
<th>29.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head space unit 1</td>
<td>39.7 ± 0.6a</td>
<td>40.0 ± 0.6a</td>
<td>41.1 ± 1.1a</td>
<td>52.2 ± 2.0a</td>
</tr>
<tr>
<td>Head space unit 2</td>
<td>39.9 ± 0.5a</td>
<td>39.5 ± 0.8a</td>
<td>41.0 ± 0.7a</td>
<td>51.2 ± 1.1a</td>
</tr>
<tr>
<td>CO2 probe analyzer</td>
<td>41.2 ± 0.6a,b</td>
<td>45.4 ± 0.9b</td>
<td>42.9 ± 1.4b</td>
<td>55.7 ± 0.7b</td>
</tr>
<tr>
<td>Titrimetric method</td>
<td>46.6 ± 0.6a,b</td>
<td>51.0 ± 0.5a</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Barometric pressure (mm Hg)</td>
<td>750.0</td>
<td>750.0</td>
<td>751.0</td>
<td>751.3</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>16.6</td>
<td>17.0</td>
<td>17.6</td>
<td>18.8</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>10.1</td>
<td>9.7</td>
<td>8.4</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Each data point represents the means ± the standard deviation of three replicates. ND = no data was collected as titrimetric endpoint was unattained.

Table 3

| Summary of Test Series III environmental conditions for each Trial of varying salinity and CO2 concentrations. |
|---|---|---|---|---|---|---|---|---|
| Trial | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| DC concentration (mg/L) | Low | Med | High | Low | Med | High | Low | Med | High |
| BP (mm Hg) | 749.6 | 741.7 | 742.7 | 744.5 | 745.2 | 746.3 | 739.2 | 740.3 | 741.3 |
| pH—meter 1 | 7.13 | 6.79 | 6.58 | 7.40 | 6.93 | 6.74 | 7.37 | 6.88 | 6.74 |
| meter 2 | 7.15 | 6.84 | 6.62 | 7.47 | 7.00 | 6.80 | 7.44 | 6.96 | 6.81 |
| Salinity (ppt) or total dissolved solids (g/L) | 0.5 | 0.5 | 0.6 | 15.2 | 15.2 | 15.2 | 30.2 | 30.1 | 30.1 |
| Temperature (°C) | 24.9 | 24.7 | 24.6 | 24.8 | 25.1 | 25.5 | 25.6 | 26.3 | 26.5 |
| DO (mg/L) | 7.6 | 7.5 | 7.4 | 7.9 | 7.7 | 7.6 | 8.2 | 8.0 | 7.6 |
| Total alkalinity (mg/L) | 122 | 208 | 261 |
all the different salinity trials except at the lowest salinity, 4.5 ppt. No titrimetric measurements were made at the 15.6 and 29.8 ppt salinity trials because a reasonable titrimetric endpoint could not be obtained and CO₂ determination at the lower salinity levels was greater than that of the HSU and CO₂ probe.

3.3. Test Series 3

Table 3 summarizes the water quality conditions during Test Series 3. The temperature during the trials increased slightly and ranged from 24.9 °C in Trial 1 to 26.5 °C at the last trial. Alkalinity also increased with the increasing salinity concentration ranging from 122 mg/L as CaCO₃ in freshwater to 261 mg/L CaCO₃ in the high salinity trial, 30.1 ppt. The results for the five analytical methods used to determine the test DC concentration for low, medium, and high DC concentrations are presented in Table 4. In the freshwater and the medium and high salinity level trials, the coefficient of variation for measuring the precision of the HSU readings averaged 3.39%, which was higher than that observed in Test Series 1 and 2. Differences between CO₂ readings of HSU 1 and 2 were not significant (P > 0.05) at the different salinities or DC ranges. Statistically significant differences did exist between the results of the CO₂ probe and those of the HSU in 5 out of the 9 test scenarios and the CO₂ probe readings, on average, were higher than HSU estimates. Medium and high salinity tests of the indirect pH method resulted in different CO₂ concentration between each other potentially due to calibration drifting or sample location within the reservoir. At each of the three DC levels tested in the freshwater trials the indirect pH method estimates of CO₂ were significantly higher than the HSU estimates and in 2 of the 3 cases significantly different from each other due to the difference in the pH measured values. The CO₂ values obtained from the nomographic, titrimetric, and the calculated from total alkalinity and pH values were also significantly higher than the HSU CO₂ measurements made in freshwater at the three different DC concentrations.

3.4. Test Series 4

In Test Series 4 HSU DC estimates were compared with DC estimates based on titration to determine titration errors related to salinity above freshwater levels. In this test series DC based on the HSU averaged 30.8 mg/L (SD = 0.2) over the range of salinity tested (5.0, 10.7, 15.5, 20.5, 25.5, and 32.7 ppt). The resulting data for this test series is presented in Table 5. For the freshwater comparison, t means of pair-wise estimates were not significantly different (P > 0.05) and averaged 32.6 mg/L (SD = 0.57) for the HSU and 30.5 mg/L (SD = 1.83) for the titration method. Differences between HSU and titration based estimates of DC increased with increasing salinity and reached a maximum of 17.4 mg/L at 32.2 ppt. These differences were statistically significant (P < 0.05) at all salinity levels. The CV for the CO₂ results with the HSU was similar to the CV of the HSU in the other test series and averaged 2.46 ± 0.71. The CO₂ probe readings, on average, were higher than HSU estimates and in 2 of the 3 cases significantly different from each other due to calibration drifting or sample location within the reservoir. At each of the three DC levels tested in the freshwater trials the indirect pH method estimates of CO₂ were significantly higher than the HSU estimates and in 2 of the 3 cases significantly different from each other due to the difference in the pH measured values. The CO₂ values obtained from the nomographic, titrimetric, and the calculated from total alkalinity and pH values were also significantly higher than the HSU CO₂ measurements made in freshwater at the three different DC concentrations.

4. Discussion

The only method that provided results that were in agreement with the head space unit under various conditions of salinity and DC concentration was the OxyGuard CO₂ probe analyzer. The price of the IRGA used for obtaining the pCO₂ percent from the HSU and the price of the OxyGuard CO₂ probe are close, $2495.00 and $2285.00 respectively. However, the calibration time for the CO₂ probe at each salinity level and to adjust to changes in CO₂ concentrations was lengthy, approximately 60 and 20 min respectively. Sampling
from the head space unit with the IRGA can be conducted after 5 min of flow through the unit and does not require recalibration with changing salinity or gas concentration. The CO₂ probe can be setup for continuous monitoring and logging (additional $600.00), it is portable, and can measure dissolved CO₂ concentration directly not requiring further calculations of the obtained value. Continuous monitoring and data logging with the HSU has also been demonstrated by Watten et al. (2004). Gas analyzers like those used onboard research vessels for oceanic carbon studies cost at a minimum of $3700.00, which does not include ancillary equipment (power supply, cleaning kit, filters, pumps) and calibration kits. CEA Instruments offers a less expensive hand held CO₂ gas monitor in the 0–1% range (<15 mg/L CO₂ depending on temperature and salinity) without the auxiliaries of the GD-888 series for under $1000.00 (CEA Model 700).

It should be noted that both the OxyGuard CO₂ probe and IRGA measure the free dissolved CO₂ in the systems and is not affected by carbonates or other dissolved substances. However, it is the CO₂ gas partial pressure that affects the fish as the diffusion of CO₂ across the gills is set by the difference in pCO₂ between the blood and water. Thus, pCO₂ and DC are intimately related. The CO₂ probe and head space unit with the IRGA both provide simple and direct measurements of the amount of CO₂ in the water that will affect the fish. Test kits, detection of pH and alkalinity changes, and titrimetric analysis are subject to disturbances from the dissolved substances found in aquaculture and seawater and limit the precision and accuracy for total CO₂ determinations (Bradshaw and Brewer, 1988; APHA, 1998; Bandstra et al., 2006). In particular, with the titrimetric method for CO₂ analysis there can be a large loss in the free CO₂ gas during sample collection, transit, and analysis. Thus, samples should be analyzed immediately after collection to minimize the effect of CO₂ changes. Errors can also be encountered by the addition of excess indicator solution for the endpoint determination or the lack of a calibrated pH meter for pH endpoint determination. Determination of CO₂ concentration by the indirect pH method (Method 5) is sensitive to the pH probe performance and location. Slight changes in pH readings due to a drifting calibration or sample locale can have a substantial effect in the calculated CO₂ concentration as evident in the results provided from the two pH meters used in this study. Small changes in pH are important because pH is a logarithmic scale. When the water is very hard, such as the water used in this study, a small change in pH can mean a substantial change in the calculated CO₂ concentration.

It is possible to build your own head space unit for dissolved CO₂ measurements in aquaculture applications that does not require sophisticated components and machine precision construction. The head space unit is constructed from readily available parts which are resistant to saltwater corrosion and the brass interior fittings are easily replaceable. The main unit cost is the infra-red gas analyzer, which is a portable, rechargeable unit that can be purchased with or without the data logging capabilities. The 0–10% CO₂ range for the IRGA it is recommended to measure DC concentration levels in typical high density production systems. The IRGAs with a DC range of 0–1% should be sufficient for systems where CO₂ levels are expected to be fairly low, i.e., hatchery or nursery systems, broodstock holding units, or quarantine facilities. The head space unit with the IRGA is a satisfactory method for measuring the total dissolved carbon dioxide in a flowing stream of seawater with the precision and accuracy required to maintain the culture of fish in an aquaculture system. In time and with experience, the aquaculturist should be able to predict upper levels of CO₂ concentration for healthy fish culture based on loading densities, feed rates, flow rates, and other variables once a number of readings have been obtained. The head space unit and CO₂ probe thus allows the worker to quickly recognize irregular functions of their RAS as indicated by high CO₂ readings due to any number of causes such as pump failure, too high a density, poor water flow, inadequate degassing, etc., and provides them a tool to measure and respond accordingly.

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References


<table>
<thead>
<tr>
<th>Table 5</th>
<th>Test Series IV comparison of dissolved CO₂ measurement (DC) with the head space units and titrimetric method at different salinities ranging from 0.3 to 32.7 ppt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (ppt)</td>
<td>Head Space Unit DC determination</td>
</tr>
<tr>
<td>0.3</td>
<td>32.6 ± 0.6a</td>
</tr>
<tr>
<td>5.0</td>
<td>32.8 ± 0.8a</td>
</tr>
<tr>
<td>10.7</td>
<td>30.0 ± 0.9a</td>
</tr>
<tr>
<td>15.5</td>
<td>27.1 ± 1.1a</td>
</tr>
<tr>
<td>20.5</td>
<td>29.7 ± 0.6a</td>
</tr>
<tr>
<td>25.5</td>
<td>32.7 ± 0.6a</td>
</tr>
<tr>
<td>32.7</td>
<td>30.3 ± 0.7a</td>
</tr>
</tbody>
</table>

Each data point represents the means ± the standard deviation of three replicates. Data in the same column with different letters differ significantly (P<0.05).


