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Direct measurement of pCO_2 in cultures of marine phytoplankton: how good is the estimate from pH_{NBS} and single point titration of alkalinity?

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ABSTRACT: In physiological studies of marine phytoplankton in culture, the CO_2 equilibrium is typically calculated from measurement of pH_{NBS} (calibrated in dilute National Bureau of Standards buffers) and a single point titration of alkalinity (A_T). This approach has widespread appeal because it is simple, inexpensive, and requires very low sample volumes. However, its continued application ignores advances in analytical and theoretical oceanic CO_2 chemistry that suggest fundamental flaws in the assumption $pH_{NBS} = -\log[H^+]$ as a consequence of the high ionic strength of seawater and liquid residual junction errors in the electrode. Here we compare directly measured pCO_2 in sterile artificial seawater with calculated values, adopting the usual assumptions and commonly used equilibrium constants. Calculated values either overestimated (ca +15 to +23% error) or underestimated (–9 to –11% error) directly measured pCO_2 depending upon whether constants were derived, respectively, on the pH_{NBS} or 'seawater' (pH_{SWS}) scales. With the currently accepted equilibrium constants on the 'total hydrogen ion' pH scale (pH_{TOT}), and converting pH_{NBS} to pH_{SWS} using an apparent activity coefficient f_H (optimum value 0.85) and then to pH_{TOT}, excellent agreement was achieved between calculated and measured pCO_2, both in sterile seawater and in cultures of the diatom *Thalassiosira pseudonana*. However, f_H is generally unknown and is specific to electrode and electrode condition, making calculated pCO_2 a rather nebulous concept. Without knowledge of f_H, calculated pCO_2 had a total uncertainty of an order (~120 ppmv at atmospheric equilibrium 360 ppmv) similar to the variation in atmospheric pCO_2 between glacial periods and the present (~160 ppmv). This method therefore clearly lacks the resolution required to address the biogeochemical significance of key physiological questions. Future studies should measure pCO_2 directly, and then if required calculate $CO_{2(aq)}$ from pCO_2 and the solubility coefficient. Alternatively, since analytical precision of calculated pCO_2 was excellent, and accuracy is potentially good when f_H is known, we advocate improved interdisciplinary collaboration in order to improve this pH & A_T approach as a simple but effective tool for the study of marine phytoplankton physiology under controlled conditions.

KEY WORDS: Dissolved carbon dioxide · Marine phytoplankton · Cultures · pCO_2 · pH · Alkalinity · Total CO_2

INTRODUCTION

Almost a quarter of a century ago, Hansson (1973a) made the following simple statement: 'The pH-value of the seawater sample obtained by this procedure is not a measure of the concentration of H^+ or the activity of H^+... it is just a value read on the pH-meter'. Hansson was referring to the calibration of pH electrodes in National Bureau of Standards (NBS) buffers and subsequent determination of pH of a sample of seawater. However, Hansson (1973a) was certainly not the first to voice such concerns (see Bates 1963, Spencer 1965). Consequently, within the marine chemistry community the use of pH as an observable in the calculation of the CO_2 equilibrium fell from favour for some time (Dickson 1993a). Subsequently, marine chemists devoted considerable efforts towards establishing meaningful scales for the determination and interpretation of pH of seawater (Dickson 1984, 1993a, b, Byrne & Breland 1989, Millero et al. 1993a) such that pH measurements are now approaching the resolution and accuracy required of a reliable observable of the CO_2 system

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(Dickson 1993a). In contrast, almost all studies involving the calculation of the CO_2 equilibrium in cultures of marine phytoplankton continue to calibrate electrodes in NBS buffers and then lean on the cornerstone assumption that $pH = -\log(a_H) = -\log[H^+]$ (where a_H is the activity and $[H^+]$ the concentration of hydrogen ions). It appears that the message of Hansson (1973a), and of many studies before and since, has been overlooked, perhaps because of the complex technical language of CO_2 chemistry, which may not lend itself to effective communication and dissemination of information to other disciplines.

In parallel with the improved understanding of seawater pH, major improvements have been made in recent years in the theoretical and analytical chemistry of the dissolved CO_2 equilibrium (see DOE 1994) such that estimation of the contribution of physical and chemical processes to the global carbon cycle is becoming relatively reliable. The role of biological processes remains less certain and is complicated by a number of contentious issues. For example, despite high concentrations (2100 µmol kg^{-1}) of total dissolved inorganic carbon (C_T) in seawater, it has been proposed that the growth rate of marine phytoplankton might be limited by diffusional transport of free CO_2 in the boundary layer around the cell (Riebesell et al. 1993). It has also been suggested that ancient oceanic pCO_2 could be hindcast from the record of the stable isotope ^{13}C in particulate organic carbon (POC) preserved in oceanic sediments (Rau et al. 1989, Freeman & Hayes 1992) since there is some evidence that bulk $\delta^{13}C$ of phytoplankton could be a proxy for ambient pCO_2 during growth (Laws et al. 1995). A fundamental question which relates to both of these proposals is whether marine phytoplankton have direct access to the relatively large pool of dissolved bicarbonate (HCO_3^-) in the ocean. Evidence for utilisation of HCO_3^- is extensive in carbon limited cultures (Burns & Beardall 1987, Merrett 1991, Raven & Johnston 1991, Colman & Rotatore 1995), but equivocal at contemporary ambient oceanic pCO_2 (e.g. Raven 1993). These questions are spawning an increasing number of controlled contemporary studies on the influence of uptake of inorganic carbon both on the dissolved CO_2 equilibrium and on isotopic composition of phytoplankton POC (e.g. see Fry 1996 for review). In order that information from these studies be meaningfully extrapolated to oceanic conditions, it is imperative to establish that analyses of the dissolved CO_2 system in culture are precise and accurate, and thus consistent with those made in the open ocean.

A fundamental problem with the dissolved CO_2 equilibrium is that neither the concentration of free carbon dioxide $[CO_2]$ (square brackets refer to concentration; $[CO_2]$ represents $CO_{2(aq)} + H_2CO_3$) nor the concentration of bicarbonate $[HCO_3^-]$ can be directly measured, but must instead be derived indirectly from other measurable parameters. Assuming equilibrium conditions, it has long been accepted (e.g. Skirrow 1975) that all components of the CO_2 equilibrium can be calculated with knowledge of any 2 of the 4 measurable parameters C_T, pCO_2, pH and total alkalinity (A_T). This procedure also requires temperature (T), salinity (S), solubility coefficient (K_0) for CO_2 (Weiss 1974), and equilibrium constants (summarized in DOE 1994) for carbonic acid (K_1 and K_2), boric acid (K_B), water (K_W) and minor bases. Alternatively, $[CO_2]$ can be calculated more directly from measured pCO_2 and K_0 (i.e. using Weiss 1974); this involves less uncertainty than the calculation described above. Recent improvements in both analytical and theoretical aspects of CO_2 chemistry (see DOE 1994) have considerably improved precision and accuracy of both measured and calculated values for pCO_2, as for other components of the dissolved CO_2 equilibrium.

These contemporary analytical approaches to oceanic CO_2 chemistry do have a number of logistical limitations however; they are expensive, involve considerable technical expertise and require relatively large sample volumes. In CO_2-related studies in cultures of marine phytoplankton, the requirement to vary C_T often dictates the use of artificial seawater, or seawater initially stripped of inorganic carbon, and so sample volume can become an important consideration. Thus high precision oceanic techniques have not always been appropriate (or even accessible) to studies involving routine, regular sampling from limited volume cultures of marine phytoplankton in artificial seawater. In consequence, calculated values for pCO_2, $[CO_2]$ or $[HCO_3^-]$ in cultures have not benefitted from recent improvements, and generally continue to depend upon somewhat dated and rather questionable foundations, usually being derived from rather simple measurements of pH & A_T. To our knowledge, direct measurements of pCO_2 in culture have not been made, or are rare. Of more concern is the absence of published estimates for precision or accuracy of values for pCO_2 or $[CO_2]$ calculated from these simple measurements. This pH & A_T approach is typically based upon some variation of the method of Strickland & Parsons (1972) where pH is determined using buffers calibrated on the NBS scale, and A_T is determined using a simple single point titration with dilute hydrochloric acid (i.e. based upon Anderson & Robinson 1946). Calculations either utilise simple tabulated values (e.g. Strickland & Parsons 1972) or can be improved by including T and S dependence of K_1 and K_2 (e.g. Edmond & Gieskes 1970, Mehrbach et al. 1973) derived on the pH_{NBS} scale. These calculations may or may not include corrections for boric acid dissociation

(i.e. constants given by Edmond & Gieskes 1970), and rarely include correction for dissociation of water or minor bases (i.e. as described by DOE 1994). Attempts are sometimes made to 'improve' calculations by using more 'up-to-date' constants for K_1 or K_2 (i.e. those of Hansson 1973b, Dickson & Millero 1987, Goyet & Poisson 1989, Roy et al. 1993a) or K_B (Hansson 1973b, Dickson 1990, Roy et al. 1993b) in combination with pH_{NBS} values. However, the above equilibrium constants were not in fact derived on the pH_{NBS} scale and are thus inappropriate for use with pH_{NBS} values.

The concept of pH_{NBS} measurements in seawater has been fundamentally criticized by marine chemists because of the assumed relationship:

$$pH = -\log(a_H) = -\log[H^+]$$

This relationship is purely notional, however, since a_H cannot in fact be measured (Dickson 1993a). Moreover, activity and concentration are related by an activity coefficient f_H such that:

$$a_H = [H^+] \times f_H$$

and because of the ionic strength of seawater, f_H does not approximate to unity, and thus a_H does not approximate to $[H^+]$. However, this equation is a gross oversimplification, as the problem is compounded by liquid residual junction errors, so that in fact f_H is electrode specific (Dickson 1993a) and can vary with the condition of the electrode (Perez & Fraga 1987). In order to circumvent these problems, a number of pH scales have been adopted for seawater work:

the 'free' hydrogen ion scale $[H^+]_F = [H^+]$
the 'seawater' scale $[H^+]_{SWS} = [H^+]_F + [HSO_4^-] + [HF]$
the 'total' hydrogen ion scale $[H^+]_{TOT} = [H^+]_F + [HSO_4^-]$

Direct oceanic pH measurements now use potentiometric or spectrophotometric methods standardized with seawater buffers which are rigorously calibrated on the 'total' $[H^+]$ scale (Dickson 1993b, Millero et al. 1993a). The most robust contemporary approach for calculation of the CO_2 equilibrium is to use the pH_{TOT} scale in conjunction with the equilibrium constants of Roy et al. (1993a, b) derived on that scale (procedure summarized in DOE 1994). Measured C_T, A_T and H^+ are input in units of moles per kilogram solution (seawater), i.e. mol kg_{SW}^{-1}, with K_0 also derived on the mol kg_{SW}^{-1} scale (Weiss 1974).

Despite these fundamental questions regarding the validity of pH_{NBS}, even now most culture studies continue to rely on the questionable assumption that $pH_{NBS} = -\log[H^+]$. The complacency can be such that comparisons are often made of small differences in half saturation constants for growth (in terms of free CO_2, HCO_3^- or C_T) without any consideration of the uncertainties involved. Another problem is that questions relating to uptake of free CO_2 or HCO_3^- involve considerable exchange of information with the freshwater literature where uncertainties in pH_{NBS} and f_H may be less significant, or at least different, because of the lower ionic strength.

This study attempts to draw together the fields of marine CO_2 chemistry and phytoplankton physiology in order to examine the validity of calculations of pCO_2 or $[CO_2]$ from simple measurements of pH_{NBS} and A_T. Indeed there are certain advantages and valid reasons to use the pH_{NBS} & A_T method in terms of simplicity of measurements and low sample volume required. An important consideration, therefore, was whether a meaningful description of the CO_2 equilibrium could be extracted from this method despite its potential limitations. That is, could the problems with pH_{NBS} be reconciled, at least to some extent, by providing a 'working' value for f_H from which an estimation of pH_{TOT} could be derived? Measurements with the precision of oceanic analytical methods are not necessarily required, since changes in C_T and pCO_2 are relatively large in cultures, but some estimate of precision and accuracy is clearly overdue.

MATERIALS AND METHODS

General experimental. Experiments were conducted in artificial seawater of salinity 30.5‰ (Harrison et al. 1980) bubbled with air prior to experiments to achieve equilibrium between C_T and the pCO_2 of laboratory air of 400 to 450 ppmv. Artificial seawater was supplemented with nitrate (549 µM), phosphate (22 µM) and silicate (106 µM); trace elements and vitamins were supplemented according to the original recipe (Harrison et al. 1980). In order to remove free CO_2 purely by physical/chemical diffusion and dehydration of HCO_3^- to CO_2, sterile artificial seawater was bubbled with N_2 gas.

All experiments were conducted at 15°C, and those with phytoplankton used the marine diatom *Thalassiosira pseudonana* (NEPCC #58). Experiments were carried out in culture flasks effectively closed to minimize CO_2 exchange. Cultures were grown in about 1.5 l of medium in 2 l (nominal) Pyrex flat-bottomed round flasks leaving an initial headspace of about 850 ml. Experiments without phytoplankton used exactly the same medium and experimental set-up. Flasks were sealed with a solid silicone stopper; spongy silicone stoppers were shown to allow a 2-fold higher leakage of CO_2 into the flask. Three outlet ports were provided through the stopper by means of glass and Tygon tubing, and 2 of these were sealed on the outside end with self-sealing push-fit connectors to prevent gas exchange into the culture flask. One tube

extended down to a frit in the culture medium, whilst the second one simply extended into the headspace. By means of a short length of Tygon tubing (low CO_2 permeability; Cole Parmer), 2 push-fit connectors and an external peristaltic pump (Masterflex), the headspace could then be recirculated through the medium in a closed system with no invasion of outside air (other than the contents of the Tygon tubing prior to pumping — about 1 ml). This allowed equilibration of pCO_2 between medium and headspace prior to pCO_2 analysis. The third outlet port extended down into the medium on the inside, and was connected on the outside to a syringe with a short length of Tygon tubing. This allowed samples to be taken from the culture for pH, A_T and C_T, but the Tygon tubing did not come into contact with culture medium except during such sampling. Using the syringe, 25 to 30 ml was removed for immediate pH_{NBS} analysis, of which 20 ml was then pipetted into a separate vessel for determination of A_T, and where necessary 1 ml pipetted off for determination of C_T. Sampling in this manner clearly allowed a small amount of air into the flask after sampling, due to equilibration of atmospheric pressure. However, although this obviously caused a slight increase in pCO_2 and decrease in pH_{NBS}, this was subsequently removed during subsequent bubbling with N_2 or growth of *T. pseudonana*. Since equilibrium was always established before sampling, this invasion did not result in any errors either in measured or calculated parameters of the CO_2 system. Mass balance considerations suggest that in any event the total invasion of atmospheric CO_2 into the flask to replace the volume of all of the samples removed would have increased C_T by about 2 µmol kg_{SW}^{-1}, or only about 0.1%.

Measurement of pH_{NBS}. pH_{NBS} was measured using a Corning 350 pH meter with a Corning '3 in 1' combination ATC probe (ATC = automatic temperature compensation), reading to 0.001 pH units. This allowed pH to be measured immediately after sampling, the ATC probe correcting for the difference in temperature between the sample (17 to 20°C) and the buffer calibration values (calibrated at 25°C). The meter and probe were calibrated with fresh NBS buffers (Sigma) at 4.00, 7.00 and 10.00 immediately prior to each set of triplicate measurements. After each 3-point calibration, the slope as a percentage of the ideal nernst slope (59.16 mV per unit pH) was usually between 99 and 100% and always over 98%. If the slope fell below 98% the probe was re-calibrated. Calibration was conducted at room temperature (~20 to 25°C), however, the ATC probe automatically corrects for small differences in buffer calibration temperature. For example, if the meter is calibrated with buffer at 23°C, then a small adjustment is automatically made for the 2°C difference between pH of the buffer at 25°C and that at 23°C. pH measurements of the samples themselves were also corrected manually for the small increase in temperature between the *in situ* flask (15°C) and the pH determination (~17 to 20°C) using the temperature reading from the ATC probe at the moment of pH determination. This increase influences the CO_2 equilibrium and must be corrected using the appropriate equation $pH(t_2) = pH(t_1) + 0.0114\,(t_1 - t_2)$ where t_1 and t_2 are the sample and *in situ* (incubation) temperatures respectively (Gieskes 1969, Grasshoff et al. 1983, Parsons et al. 1984). Alternatively the final calculated pCO_2 could have been corrected for temperature according to the equations of Copin-Montegut (1988). These corrections ensured that pH_{NBS} measurements were of the highest possible quality; in fact, the average standard deviation of a large number of triplicate measurements of pH_{NBS} was about 0.005 after correction to *in situ* temperature. This represents a precision (% 1 σ) in $[H^+]$ of about 1.1%.

Measurement of A_T. A_T was determined according to a reduced volume version of the Strickland & Parsons (1972) method which was taken from the original method of Anderson & Robinson (1946). 20 ml of medium from the pH_{NBS} determination was pipetted into a small beaker with 5 ml of 0.01 N HCl (±1%, Fisher Scientific) and the pH was determined immediately (to 0.001 units) after careful calibration (see above). From this pH_{NBS} measurement, A_T was calculated according to the calculation given by Strickland & Parsons (1972). Average standard deviation of a large number of triplicate measurements of A_T was typically about 6 µmol kg_{SW}^{-1} representing a precision (% 1 σ) of about 0.3%. Accuracy of A_T was more difficult to define, since true calibration standards have only very recently become available (see footnotes to Table 1).

Measurement of pCO_2 and $[CO_2]$. pCO_2 was measured directly using an ADC 225 Mk 3 infra-red gas analyser (IRGA). The headspace in the culture flask was recirculated through the medium using an external pump, as described above, for about 20 min until equilibration was reached between medium and headspace (stable pCO_2 reading). This time was checked independently and agrees well with that established for standard pCO_2 methods (e.g. DOE 1994, Purdie & Finch 1994, Robertson et al. 1994). The push-fit connectors from the external pump were then disconnected from those on top of the flask and replaced with push-fits to short lengths of Tygon tubing leading to the IRGA. The internal pump of the IRGA passed the headspace gas through the analysis cell and back into the flask via the tube and frit (i.e. a closed system passing through the IRGA). The IRGA was calibrated at 0 ppmv using N_2 gas, and at 500 ppmv using a calibrated CO_2 in air mixture (Praxair). The tubing and the IRGA cell and pump were flushed with N_2 prior to each

analysis, and subsequent pCO_2 measurements were corrected for the small dilution caused by the presence of N_2 in the tubing and analysis cell (typically <5%). Since the resolution of the analogue instrument was about 10 ppmv (5 ppmv at best), the relatively small correction for atmospheric pressure (to convert ppmv to µatm) was not undertaken. pCO_2 values were reported as ppmv in moist air (as recommended by DOE 1994). The instrument is not sensitive to water vapour interference, but care was taken to ensure a temperature differential between the instrument and the culture medium to prevent condensation inside the analysis cell. Since $[CO_2]$ cannot be directly measured, 'measured $[CO_2]$' was in fact calculated from measured pCO_2 and the solubility coefficient K_0. This was the only available estimate for $[CO_2]$ which was independent of pH_{NBS} uncertainties. The standard deviation of triplicate measurements of pCO_2 was about 10 ppmv and was largely determined by the limited resolution of the analogue scale on the instrument. This represents a precision (% 1 σ) of about 3% at atmospheric equilibrium.

Measurement of C_T. When required, direct measurement of C_T was made by injecting 1 ml subsamples of culture medium through a septum into a 1 l flask flushed with N_2. 0.5 ml phosphoric acid (1 M) was then injected into the flask and the gas passed in a closed loop through the IRGA (i.e. returned to the flask) to measure liberated CO_2. The method was calibrated against $NaCO_3$ standards. The standard deviation of triplicates was about 30 µmol kg_{SW}^{-1} and as with pCO_2, this was largely determined by the resolution of the analogue scale of the instrument. This represents a precision (% 1 σ) of around 1.5%

Calculation of pCO_2, $[CO_2]$ and $[H^+]_{TOT}$ from other measurables. Calculated pCO_2 was determined from pH_{NBS} and A_T by several alternative approaches:

(1) Simple tabulated values from the conversions of Strickland & Parsons (1972).

(2) Spreadsheet calculation typifying the approach usually used for cultures of marine phytoplankton. This included T and S dependence of K_1 and K_2 of Mehrbach et al. (1973), Edmond & Gieskes (1970), Hansson (1973b), and Goyet & Poisson (1989), and the T and S dependence of K_0 on the mol l^{-1} scales (Weiss 1974). A_T was converted to carbonate alkalinity (A_C) using total boron (B_T) estimated from salinity (Uppström 1974) and the T and S dependence of boric acid dissociation (Edmond & Gieskes 1970). pCO_2 and $[CO_2]$ were calculated from $[H^+]$ and A_C according to the standard equations of the CO_2 equilibrium (Skirrow 1975, DOE 1994). This meant that the 4 approaches differed only in the estimates of K_1 and K_2. The additional choice of the constants of Hansson (1973b) and Goyet & Poisson (1989) was a deliberate one; these are in fact inappropriate for the pH_{NBS} scale, having been derived on the 'seawater pH scale' (pH_{SWS}). However, these constants were included in order to highlight and emphasise the errors encountered when attempting to 'improve' calculations from pH_{NBS} using more recent but inappropriate dissociation constants.

(3) A more refined spreadsheet was constructed utilizing all of the currently accepted constants available (DOE 1994). All equations necessary have been converted to common scales of pH_{TOT} and mol kg_{SW}^{-1} by DOE (1994). T and S dependency of K_1, K_2, K_B and K_W are given by DOE (1994) adapted from Roy et al. (1993a, b) and of K_0 from Weiss (1974) on the mol kg_{SW}^{-1} scale. Measured A_T was converted to mol kg_{SW}^{-1} using the seawater density routine of Millero & Poisson (1981) (also given by DOE 1994). An improved estimate of B_T was taken directly from Harrison et al. (1980) in order to calculate A_C from A_T using the T and S dependency of K_B (DOE 1994). pH_{NBS} was converted to pH_{SWS} (Butler et al. 1985, Whitfield et al. 1985, Dickson & Millero 1987) using $pH_{SWS} = pH_{NBS} + \log f_H$ and a range of values for f_H between 0.75 and 1.0 (Mehrbach et al. 1973, Butler et al. 1985, Whitfield et al. 1985, Perez & Fraga 1987). pH_{SWS} was then converted to pH_{TOT} with a correction for hydrogen fluoride [HF] using total fluoride F_T = 64.3 µmol kg_{SW}^{-1}, and total sulphate S_T = 24.5 mmol kg_{SW}^{-1}, taken directly from the recipe of Harrison et al. (1980), and using the T and S dependence of the equilibrium constants K_F and K_S (DOE 1994). $[H^+]$ was converted to the mol kg_{SW}^{-1} scale (Dickson 1993b, DOE 1994). pCO_2 and $[CO_2]$ were then calculated from $[H^+]$ and A_C according to the standard equations of the CO_2 equilibrium (Skirrow 1975, DOE 1994). By varying f_H in the calculation, an optimal value of f_H giving the best fit between calculated and measured pCO_2 could be estimated. When required, pH_{TOT} was calculated from A_T & pCO_2, C_T & pCO_2 and C_T & A_T according to the equations of DOE 1994). For the C_T & A_T and the A_T & pCO_2 pairs, an iterative solution for $[H^+]$ was required in order to calculate A_C from A_T using K_B, B_T and $[H^+]$. Precision and estimated uncertainty of calculated parameters (Table 1) were determined using the precision (% 1 σ) of measured parameters and equilibrium constants, together with the derivatives given by Dickson & Riley (1978).

The contribution of dissociation products of phosphoric and silicic acids to A_T was not considered in calculations of pCO_2. In cultures supplemented with high nutrients, these so called minor bases could become significant, but changes in the concentrations of total phosphorus P_T and silicon Si_T would be required for the calculation. In the cultures, an estimation of the maximum initial contribution to A_T was made from total concentrations of initial nutrients P_T and Si_T of 22

and 106 µmol kg_{SW}^{-1} respectively, using dissociation constants K_{1P}, K_{2P}, & K_{3P}, and K_{Si} (DOE 1994). This gave contributions of P and Si to A_T of about 23 and 3 µmol kg_{SW}^{-1} respectively at pH around 8, representing about 1% of A_T. This does not cause an error in A_T because this measures all contributions to alkalinity. However, there could be a small resulting error in calculated pCO_2 because A_T must be corrected to A_C for minor bases in order to calculate the CO_2 equilibrium; an error of 1% in A_T results in an error in calculated pCO_2 of 1% (Dickson & Riley 1978). Although P_T and Si_T decrease during growth of a culture, these were non-limiting and so would not decrease to zero. Calculations suggest that the increase in pH and subsequent dissociation of P_T and Si_T would offset the decrease in concentration so that the 1% contribution to A_T is approximately maintained throughout the culture.

RESULTS

The simplest calculation of pCO_2 from pH_{NBS} & A_T is to use the tables of Strickland & Parsons (1972), and the relationship between calculated and directly measured pCO_2 using this approach is shown in Fig. 1. The right hand side of the *y*-axis shows the range of free CO_2 concentration equivalent to the range of pCO_2, for the given temperature and salinity of the experiment (i.e. calculated according to Weiss 1974). For such a simple approach, measured and calculated pCO_2 are in remarkable agreement with the 1:1 relationship; however, this does not validate the use of the Strickland & Parsons (1972) tables because of problems with the electrode specificity of pH_{NBS} measurements (see Fig. 5 and 'Discussion').

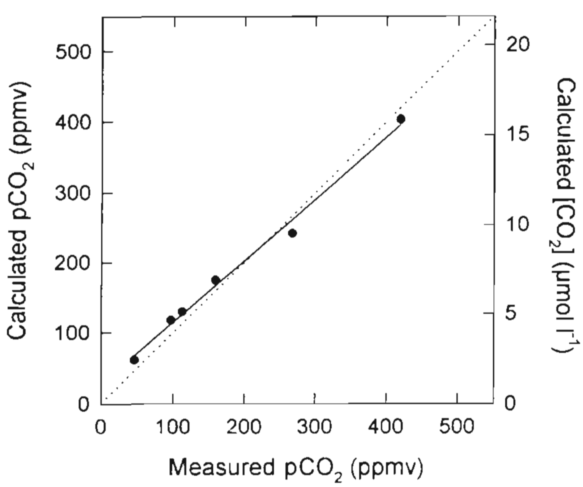

Fig. 1 Comparison of directly measured pCO_2 with values calculated from measurements of pH_{NBS} & A_T using the tables of Strickland & Parsons (1972) using units of mol l^{-1} The experiment was conducted in artificial seawater (Harrison et al. 1980) at temperature 15°C and salinity 30.5‰, with free CO_2 removed by bubbling with nitrogen gas. Dotted line represents 1:1 relationship between directly measured and indirectly calculated values of pCO_2. Solid line represents least squares regression fit to data ($r^2 = 0.992$, $p < 0.001$). Right hand *y*-axis represents the range of concentration of free CO_2 (µmol l^{-1}) equivalent to the range of pCO_2 at the given temperature and salinity of the experiment (using Weiss 1974)

The Strickland & Parsons (1972) tables are also limited in their ability to calculate low pCO_2 values encountered during the late stage of a culture, and the approach also lacks the precision required of many contemporary questions regarding dissolved CO_2 in cultures. Improvement upon these tabulated values required a breakdown of the theoretical basis behind the calculations, and the Strickland & Parsons (1972) tables do not give this in detail. In order to formulate a calculation which could resolve small changes in pH, and thus pCO_2 or $[CO_2]$, and one which could handle very low values of pCO_2 at pH values beyond the range of the Strickland & Parsons (1972) tables, the calculations for pCO_2, $[CO_2]$ & C_T from pH & A_T (Skirrow 1975) have been utilized by many authors. Here we adopted this approach using several alternative estimates of the equilibrium constants K_1 and K_2 commonly utilized: those of Edmond & Gieskes (1970) and Mehrbach et al. (1973), both derived on the pH_{NBS} scale and commonly used with pH_{NBS} measurements, and the constants of Hansson (1973b) and Goyet & Poisson (1989), both derived on the pH_{SWS} scale.

The same 6 pairs of pH_{NBS} & A_T measurements as shown in Fig 1 were treated with the 4 different calculation routines described in the 'Materials and methods' (Fig. 2). The 4 routines used the same equations and assumptions, but varied the constants for K_1 and K_2. The constants of Mehrbach et al. (1973) and Edmond & Gieskes (1970) gave calculated values for pCO_2 which were similar, but with an error of the order of +15 to +23% of the directly measured values. In contrast, the constants of Hansson (1973b) and Goyet & Poisson (1989) gave calculated values with an error of the order of -9 to -11% of measured values.

To optimize the calculation in accordance with potentially the most precise and accurate constants available, pH_{NBS} was converted to pH_{TOT} using f_H as outlined in the 'Materials and methods' section, in order to utilize the constants derived on the pH_{TOT} scale (see DOE 1994; constants adapted from Roy et al. 1993a, b). The calculation was performed with a choice of 3 values for f_H of 1, 0.85, and 0.75. The value $f_H = 1$ corresponds to the situation where activity and concentration are equal, and as would be expected calculated pCO_2 corresponded approximately with that derived using the Goyet & Poisson (1989) constants (Fig. 2). However, this calculated value was then

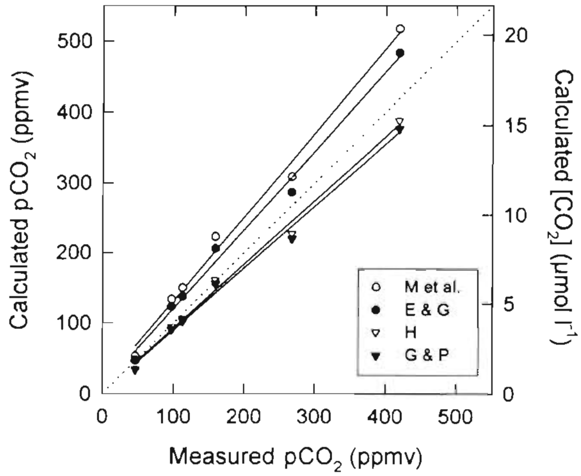

Fig. 2. Comparison of directly measured pCO_2 with values calculated from the same pairs of data as shown in Fig. 1. pCO_2 was calculated from measurements of pH_{NBS} & A_T using units of mol l^{-1}, the equilibrium constants of Mehrbach et al. (1973), Edmond & Gieskes (1970), Hansson (1973b) and Goyet & Poisson (1989), and assuming $pH = -\log a_H = -\log[H^+]$. Solid lines represent least squares regression fits to data (all $r^2 > 0.99$, all $p < 0.001$). Error bars smaller than symbol size; for analytical precision and accuracy see Table 1

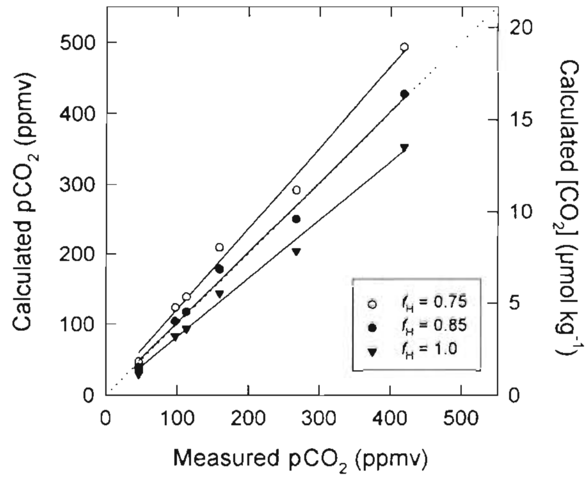

Fig. 3. Comparison of directly measured pCO_2 with values calculated from the same pairs of data as shown in Figs. 1 & 2. pCO_2 was calculated from measurements of pH_{NBS} & A_T using units of mol per kg seawater (mol kg_{SW}^{-1}), the equilibrium constants of Dixon & Goyet (1994) and converting pH_{NBS} to pH_{TOT} using a range of values for the apparent activity coefficient f_H of 0.75, 0.85 and 1.0. Right hand y-axis represents the range of concentration of free CO_2 (µmol kg_{SW}^{-1}) equivalent to the range of pCO_2 at the given temperature and salinity of the experiment (using Weiss 1974). Solid lines represent least squares regression fits to data (all $r^2 > 0.99$, all $p < 0.001$). Error bars smaller than symbol size; for analytical precision and uncertainty see Table 1

improved to give excellent agreement between calculated and measured pCO_2 when f_H was 'tuned' to an optimum value of around 0.85 (Fig. 3). Using the value for $f_H = 0.75$, taken approximately as a 'typical' value for the given T and S of these experiments (e.g. Mehrbach et al. 1973, Perez & Fraga 1987), the calculated pCO_2 again became a significant overestimate, approximating the estimate of pCO_2 derived using the constants of Mehrbach et al. (1973).

Clearly this method optimized the value for f_H as the one giving the best fit between calculated and measured pCO_2, and so could have been simply fortuitous rather than representing a realistic value for f_H. Taking a rather different approach, this value of f_H was checked independently. The calculation for pCO_2 from pH_{TOT} & A_T was modified so that pH_{TOT} could be calculated either from pCO_2 & A_T, from pCO_2 & C_T or from C_T & A_T (e.g. Skirrow 1975, DOE 1994). These 3 pairs of measurables are independent of f_H and so were utilized to check the value of pH_{TOT} converted from pH_{NBS} using $f_H = 0.85$ (i.e. 'measured' pH_{TOT}). Fig. 4 confirmed that pH_{TOT} calculated from pH_{NBS} using $f_H = 0.85$ indeed gave excellent agreement with values for pH_{TOT} calculated independently from the other measurables.

However, when this approach was repeated this 'optimum' value for f_H varied with the same electrode over time, consistent with the observation that liquid residual junction errors are electrode specific and vary

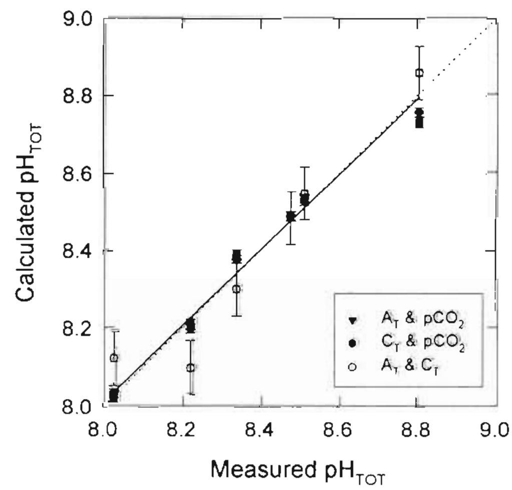

Fig. 4. Comparison of 'measured' pH_{TOT} with pH_{TOT} calculated from pairs of other measurables of the CO_2 equilibrium from the experiment shown in Figs. 1, 2 & 3. The pairs of measurables A_T & pCO_2, C_T & pCO_2, and A_T & C_T are independent of f_H. 'Measured' pH_{TOT} was in fact calculated from directly measured pH_{NBS} using $f_H = 0.85$. Error bars represent analytical uncertainty ($\pm 1\ \sigma$) of calculated pH_{TOT} derived according to Dickson & Riley (1978) for the 3 sets of measurable pairs (see Table 1 for details). Solid line represents least squares regression fit through all data ($r^2 = 0.96$, $p < 0.001$)

with age of electrode (Whitfield et al. 1985, Perez & Fraga 1987, Dickson 1993a). A simple experiment confirmed this; a new identical electrode was bought when the original electrode was about 15 mo old. The response of the 2 electrodes was compared instantaneously using the twin channels of the pH meter. The old and new electrodes were simultaneously calibrated in separate beakers of NBS buffers of pH 4.00, 7.00 and 10.00, and then placed back in buffers of pH 7.00. The data logger was then switched on and the 2 electrodes placed into 2 separate beakers of air-equilibrated artificial seawater for about 15 min, before being placed back into buffer of pH 7.00. The responses of the 2 electrodes (Fig. 5) suggest that despite calibrating identically in pH 7.00 buffer, both before and after placement in seawater, the electrodes gave quite different values for pH_{NBS} of the seawater sample. Also of note is the time taken for the pH_{NBS} reading to stabilize after placing in seawater; Strickland & Parsons (1972) recommended waiting for 5 min, but we would clearly recommend at least 10 min (Fig. 5). For routine measurements, a more effective solution was employed: the electrode was placed in artificial seawater after calibration, so that when the samples were ready, the probe had already stabilized in the seawater for at least 15 min. This both eliminated stabilization time and, because of this, reduced contact time of the sample with the atmosphere to about 1 min, suggesting that atmospheric exchange of CO_2 during measurement of pH was negligible.

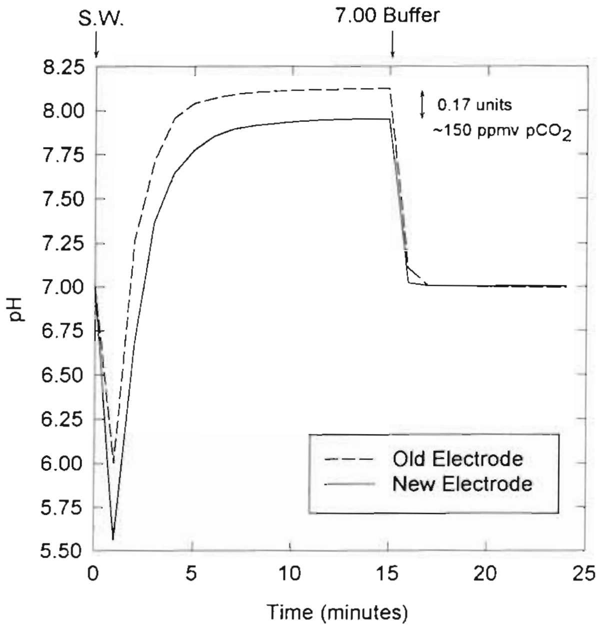

Fig. 5. Time response of pH_{NBS} measurements of air-equilibrated seawater using 2 identical probes of different ages. Both probes were first calibrated in separate beakers of NBS buffers of pH 4.00, 7.00 and 10.00, and then placed in separate beakers of pH 7.00 buffer. The data-logger was then switched on and both probes placed simultaneously in separate subsamples of air-equilibrated seawater for about 15 min, before being returned to the buffer. Despite having exactly the same calibration pH in dilute buffer, the probes show an enormous discrepancy in the final stabilized pH_{NBS} of the seawater. The resulting uncertainty in calculated pCO_2 would be around 150 ppmv at $pH_{NBS} \approx 8.000$. Initial decrease in pH was probably the result of the wash of distilled water prior to measurement

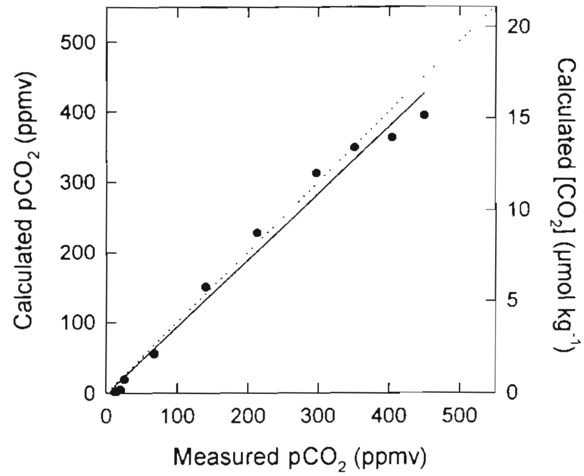

Fig. 6. Comparison of directly measured pCO_2 with values calculated using the same approach as shown in Fig. 3, but in this case free CO_2 in the artificial seawater was depleted by a growing culture of the marine diatom *Thalassiosira pseudonana*. pCO_2 was calculated from measurements of pH_{NBS} & A_T and pH_{NBS} converted to pH_{TOT} using f_H = 0.85. Solid line represents least squares regression fit to data (r^2 = 0.984, $p < 0.001$). Error bars smaller than symbol size; for analytical precision and uncertainty see Table 1

The same approach as the experiment in Fig. 3 was repeated using cultures of the marine diatom *Thalassiosira pseudonana*, and confirmed that this worked equally well when removal of C_T was effected by a marine phytoplankter (Fig. 6) rather than by bubbling with N_2. With the same assumed value of f_H = 0.85, excellent agreement was achieved between calculated and measured pCO_2 (Fig. 6). However, there were some minor discrepancies between calculated and measured values, particularly after inoculation and also towards stationary phase.

DISCUSSION

In the ensuing discussion, reference to pCO_2 can equally be directed to $[CO_2]$ as at constant temperature this is proportional to pCO_2 through the solubility coefficient K_0 (Weiss 1974). Predicted pCO_2 derived from the tables of Strickland & Parsons (1972) gave surpris-

ingly good agreement with the measured values (Fig. 1) when compared to the poor agreement observed between calculated and measured values (Fig. 2) using the constants of Edmond & Gieskes (1970) and Mehrbach et al. (1973). It is conceivable that the Strickland & Parsons (1972) approach was based upon some sort of empirical correction factor, thus explaining the observed good agreement. However, when Fig. 5 was also considered, it became clear that the degree of agreement observed (Fig. 1) was in fact electrode dependent, and that another electrode could equally have given poor agreement using the Strickland & Parsons (1972) tables. Electrode dependent uncertainty in measured pH_{NBS} (Fig. 5) was of the order of 0.17 units, and could even be greater under certain circumstances. In terms of accuracy, therefore, this surely negates the use of the Strickland & Parsons (1972) tables for calculation of pCO_2. In any event, this approach suffers from the limited range and lack of precision of the tabulated pH values.

Calculated pCO_2 using the constants of Edmond & Gieskes (1970) and Mehrbach et al. (1973) was of the order of a 15 to 23% overestimate of measured pCO_2 (Fig. 2), an error well in excess of typical analytical precision and estimated uncertainty (Table 1). Calculated pCO_2 was significantly changed, but in terms of accuracy, barely improved by utilizing the constants of Hansson (1973b) and Goyet & Poisson (1989), which resulted in underestimates in calculated pCO_2 of the order of 9 to 11%, again in excess of analytical precision and estimated uncertainty (Table 1). The differences between calculated and measured pCO_2 using any of the equilibrium constants demonstrates invalidity of this approach from pH_{NBS} and A_T when assuming $pH = -\log[H^+]$. Although the 'within method' precision (i.e. using one set of constants) was good (see Table 1), the precision 'between methods' using the range of constants was very poor, as indeed was the potential accuracy (Fig. 2). The calculated pCO_2 derived using the constants of Mehrbach et al. (1973) was about 34% higher than that derived using the constants of Goyet & Poisson (1989). However, when the constants of Mehrbach et al. (1973) are adjusted to the pH_{SWS} scale, it has been shown that they agree well with those of Hansson (1973b) and Goyet & Poisson (1989) derived on that scale (Dickson & Millero 1987, Goyet & Poisson 1989).

Table 1. Summary of analytical precision and estimated uncertainty in measured and calculated parameters. Errors calculated using precision (% 1 σ) of measured parameters and the derivatives presented by Dickson & Riley (1978)

Analytical precision (% 1 σ) of measured parameter							Derived parameter	Measured pair	Combined error (% 1 σ) of derived parameter	
C_T	pCO_2	$[H^+]_{NBS}$	A_C	K_0	K_1	K_2			Precision[a]	Uncertainty[b]
–	–	1.1[c] (2.3)	0.3[d] (0.6)	0.5[e]	2.3[e]	4.6[e]	pCO_2[f]	pH_{NBS} & A_T	1.4	3.8
–	–	1.1[d] (2.3)	0.3[d] (0.6)	0.5[e]	0.5[g]	0.7[g]	pCO_2[h]	pH_{NBS} & A_T	1.4	2.9
1.5	3.0	–	–	0.5[e]	0.5[g]	0.7[g]	$[H^+]_{TOT}$	C_T & pCO_2	3.0	3.1
–	3.0	–	0.3[d] (0.6)	0.5[e]	0.5[g]	0.7[g]	$[H^+]_{TOT}$	A_C & pCO_2	2.5	2.6
1.5	–	–	0.3[d] (0.6)	–	0.5[g]	0.7[g]	$[H^+]_{TOT}$	A_T & C_T	16.4	17.1

[a] Precision of derived parameter uses the combined errors of the measured pair only (Dickson & Riley 1978)

[b] Uncertainty of derived parameter takes into account errors in the measured pair and in the equilibrium constants (Dickson & Riley 1978)

[c] First value represents % 1 σ of triplicates of pH and is used for combined precision. Value in parentheses used for combined uncertainty and represents the uncertainty in pH of 0.01 resulting from resolution of buffer calibration

[d] First value represents % 1 σ of triplicates of A_T and is used for combined precision. Value in parentheses used for combined uncertainty and represents analytical uncertainty of A_C based upon uncertainties in the correction of A_T to A_C using $[H^+]$ and K_B (see Dickson & Riley 1978). The real accuracy of the single point A_T titration has been difficult to establish because 'true' calibration standards for A_T have only just become available (Andrew Dickson pers. comm.). Preliminary comparisons (unpubl.) with potentiometric titrations indicate accuracy of better than 1% (in agreement with value of 0.6% quoted above from Dickson & Riley 1978), assuming of course that the potentiometric titration was reasonably accurate

[e] Taken from Dickson & Riley (1978)

[f] Represents pCO_2 as calculated for Fig. 2

[g] Taken directly from Roy et al. (1993a). These constants were selected for the DOE (1994) handbook on the basis that they were the most precise values published, and because they were derived by a method giving potentially the most accurate results (Andrew Dickson pers. comm.). This however does not necessarily ensure that they are the most accurate, and the likely range of error between all sets of published constants is about 0.015 in pK_1 and 0.03 in pK_2, or about 2.5% and 3.3% respectively (Andrew Dickson pers. comm.)

[h] Represents pCO_2 as calculated for Fig. 3 but uncertainties resulting from f_H not included here — see text

We conclude therefore, for reasons discussed later, that it is the misuse of constants derived both on the pH_{NBS} scale (Edmond & Gieskes 1970, Mehrbach et al. 1973) and on the pH_{SWS} scale (Hansson 1973b, Goyet & Poisson 1989), rather than significant errors in the constants themselves, that results in the observed poor agreement between, and accuracy of, calculated pCO_2 values (Fig. 2). The scale of the imprecision and inaccuracy due to misuse of constants was approximately an order of magnitude greater than the analytical precision and uncertainty of the method itself.

In order to rectify this poor agreement and inaccuracy, the next calculation (Fig. 3) included the constants and equations recommended by DOE (1994), as outlined in the methods section. Assuming $f_H = 1$, calculated pCO_2 (Fig. 3) approximated the underestimate given using the Goyet & Poisson (1989) constants (Fig. 2). This was to be expected as there is little difference between the Goyet & Poisson (1989) constants derived for the pH_{SWS} scale and the constants of DOE (1994) derived for the pH_{TOT} scale. Forcing $f_H < 1$ resulted in an increase in calculated pCO_2 up to an optimum point ($f_H = 0.85$) at which excellent agreement was achieved. Higher values of calculated pCO_2 were forced by using $f_H = 0.75$ so that the total uncertainty resulting from variation in f_H was of the same order as that resulting from choice of equilibrium constants (Fig. 2), and again was approximately an order of magnitude greater than the analytical precision and uncertainty (Table 1). The optimum $f_H = 0.85$ was further confirmed by calculating pH_{TOT} from pairs of other measurables which were independent of f_H (Fig. 4). The $f_H = 0.85$ value also worked well for cultures of the marine diatom *Thalassiosira pseudonana*, although, for reasons unknown, some deviation between calculated and measured pCO_2 occurred during stationary phase and just after inoculation (Fig. 6). Although the value of $f_H = 0.85$ was applicable for the particular electrode and time of application, f_H varied between electrodes (Fig. 5) and probably with age and condition of electrode (see also Perez & Fraga 1987). f_H is also known to be T and S dependent (Butler et al. 1985, Whitfield et al. 1985) and so this optimum $f_H = 0.85$ is specific to the electrode, its condition, and our experimental conditions of S = 30.5‰ and T = 15°C. Optimum f_H was higher than typical values in the literature (e.g. Mehrbach et al. 1973, Butler et al. 1985, Whitfield et al. 1985, Perez & Fraga 1987), but in our study the electrode was relatively new and experimental conditions were slightly different. Indeed, the f_H value required to optimize agreement appeared to decrease with age of this electrode (authors' pers. obs.) and the value of $f_H = 0.85$ clearly cannot be extrapolated to other situations. In retrospect, we derived f_H perhaps a little too pragmatically in this study; f_H derived in this manner could be influenced by any factors causing small errors in both calculated and measured pCO_2, and so may not represent the exact 'true' value for the given electrode and experimental conditions. We did however confirm f_H independently (Fig. 4) and therefore feel that the value is reasonably representative. Moreover, the purpose of this study was more to highlight inadequacies in the typical pH_{NBS} & A_T calculation, and in our understanding and interpretation of pH measurements in seawater, rather than to provide absolute values of f_H, or information on variation of f_H under different conditions.

The data in Fig. 2 became much clearer in the light of considerations of f_H (Fig. 3). The differences between values derived using the constants of Mehrbach et al. (1973) and those derived using Edmond & Gieskes (1970) probably resulted from smaller scale differences in f_H between the 2 studies. In contrast, the values obtained using the constants of Hansson (1973b) and those of Goyet & Poisson (1989) were in good agreement, reflecting the independence of the pH_{SWS} scale constants from consideration of f_H. The significantly greater differences in calculated pCO_2 (Fig. 2) observed between those based upon constants derived on the pH_{NBS} scale and those derived on the pH_{SWS} scale do not reflect major differences in K_1 and K_2, but are more due to the fact that these constants were derived on different pH scales. Dickson & Millero (1987) converted the constants of Merhbach et al. (1973) to the pH_{SWS} scale by dividing K_1 and K_2 by the f_H values presented by Mehrbach et al. (1973), and then found good agreement between those of Mehrbach et al. (1973) and those of Hansson (1973b). Ironically, although derived on the pH_{NBS} scale, the constants of Mehrbach et al. (1973) are not valid with pH_{NBS} measurements, such as those reported in the literature or measured in our study, unless the f_H values coincide, or unless the constants and pH values are converted to some common scale. The difference between calculated and measured pCO_2 (Fig. 2) using constants of Mehrbach et al. (1973) simply reflects the difference in $f_H \approx 0.75$ obtained in that study (value taken for similar conditions to ours) and our value of $f_H = 0.85$. If the constants of Mehrbach et al. (1973) had been adjusted to the pH_{SWS} scale using $f_H \approx 0.75$, and our pH_{NBS} measurements adjusted to the pH_{SWS} scale using $f_H = 0.85$, then good agreement should have resulted. This explains why the calculated pCO_2 with $f_H = 0.75$ (Fig. 3) is in reasonable agreement with the value calculated (Fig. 2) using the constants of Mehrbach et al. (1973). Whichever approach is used, without knowledge of f_H, comparisons must be specific to the experimental conditions and electrode, and calculation of accurate values for pCO_2 or $[CO_2]$ is highly unlikely.

We conclude that even the constants of Mehrbach et al. (1973), derived on the pH_{NBS} scale, cannot be used with pH_{NBS} measurements in order to calculate pCO_2 or $[CO_2]$. Using such a combination, accurate values could only be calculated fortuitously in the unlikely event that the f_H value for the electrode employed coincided with that of Mehrbach et al. (1973). Neither can we recommend the use of the constants of Hansson (1973b) or Goyet & Poisson (1989) with pH_{NBS} values, as this clearly gives inaccurate values; these 2 latter sets of constants were derived on the pH_{SWS} scale and are clearly inappropriate for use with pH_{NBS} measurements unless f_H is known (Fig. 3).

Most of the uncertainty arose therefore from choice and misuse of equilibrium constants (Fig. 2) and uncertainties in pH_{NBS} and f_H (Fig. 3); analytical precision and uncertainties appeared to be negligible (see Table 1) in the context of these major uncertainties. The precision of the single point A_T titration (see Strickland & Parsons 1972) is obviously poorer than that of potentiometric methods, but our value of about 0.3% (Table 1; see also Dickson & Riley 1978) is by no means excessive. Accuracy of this A_T method is harder to evaluate, and inaccuracies can arise from variations in acid strength (if not monitored) resulting from volatility, errors in electrode calibration and several other factors. However, since true A_T standards have only just become available (see footnotes to Table 1), the question of accuracy is a problem common to most A_T analyses. In any event, if uncertainty in A_T is indeed better than 1% (see Table 1), then this will contribute negligibly to the major uncertainties in calculated pCO_2 resulting from the pH_{NBS} measurement. Errors in A_T of the order of 1% only result in errors in calculated pCO_2 of around 1% (Dickson & Riley 1978). A_T does not change with removal of free CO_2 and so is not a major variable during growth of non-calcifying cultures of phytoplankton, other than the relatively small change which results from assimilation of nitrogen species (Brewer & Goldman 1976); because of this, the analytical uncertainties in A_T will not be relatively 'amplified' as a culture grows because A_T is not significantly reduced.

Measurement of pH after calibration in NBS buffers (and assumption of $pH = -\log[H^+]$) has become so acceptable a practice in physiological studies of marine phytoplankton that details of pH measurements and calibrations are often omitted in publications (e.g. Merrett 1991, Colman & Rotatore 1995, Rotatore et al. 1995, Israel & González 1996, Korb et al. 1997). In the present study, we made very careful pH measurements, and achieved an excellent precision (1 σ) of about 0.005 units for triplicate pH measurements (after temperature corrections). This was in part achieved by allowing sufficient time for stabilization of the probe (Fig. 5; also see Strickland & Parsons 1972) in seawater after calibration, but prior to measurement of samples. It was also in part achieved by very careful temperature corrections using the ATC probe. This allowed correction of pH from the temperature at which samples were measured, back to the pH at *in situ* temperature, and also allowed correction for small differences between the temperature at which buffer calibrations were made and the stated temperature at which buffer calibrations are in fact valid (25°C). The majority of studies do not describe such attention to detail in calibration and measurement of pH, and we would suggest that because of this, the real uncertainty in calculated pCO_2 or $[CO_2]$ 'between studies' is even greater than that suggested by Figs. 2 & 3. For example, not taking into account the time required for stabilization in high ionic strength medium (Fig. 5) could add an enormous uncertainty to measured pH_{NBS} values. Publications often do not give sufficient details of equilibrium constants used either, and can be unspecific in all manner of details regarding calculations of the CO_2 equilibrium. For example, many do not specify whether they refer to A_T or A_C, and thus whether corrections using K_B and B_T have been applied; erroneous use of A_T instead of A_C in calculations could result in an additional error in calculated pCO_2 of 3 to 10%. These are all fundamental details required to assess the efficacy of estimates of pCO_2 and $[CO_2]$; we advocate that future studies be much more explicit in their description of how parameters of the CO_2 equilibrium are both measured and calculated.

The application of calculations of the CO_2 equilibrium derived from pH_{NBS} measurements are extensive in the field of phytoplankton physiology. The constants of Mehrbach et al. (1973) and others, such as Riley & Chester (1971), on the pH_{NBS} scale are frequently used (e.g. Dong et al. 1993, Thompson & Calvert 1994, Colman & Rotatore 1995, Laws et al. 1997), and some have used the constants of Goyet & Poisson (1989) in combination with pH_{NBS} (e.g. Merrett et al. 1996). Other studies such as Riebesell et al. (1993) cite the approach of Grasshoff et al. (1983), without specifying which of the 2 sets of constants described by Grasshoff (i.e. Mehrbach et al. 1973 and Hansson 1973b) were actually employed, an omission which according to our Fig. 2 is quite significant. One of the few physiological studies not to use pH_{NBS} was that of Laws et al. (1995), who calculated the CO_2 equilibrium from C_T & A_T and thus avoided the uncertainties inherent with pH. We should point out that care is required when calculating the CO_2 equilibrium from these pH independent measurable pairs, such as C_T & A_T. If pH itself were to be calculated, then the relevant pH scale on which this is based should be specified, and this will depend upon the constants chosen. In the case of Laws et al. (1995),

the constants of Roy et al. (1993a) were used, and so if pH had been calculated, it would be a pH_{TOT} value and not directly comparable with pH_{NBS} values commonly quoted in the physiological literature.

pH is specified in the models of Rau et al. (1996) and Riebesell et al. (1993), seemingly with the implicit assumption $pH = -\log[H^+]$. These models show much promise in the understanding of uptake of CO_2 and isotope discrimination. However, before experimentalists begin to provide validation data for such models, some consensus and clarification is clearly required on meaningful definitions and applications of seawater pH measurements. Uncertainties in pH_{NBS} measurements not only influence the calculation of pCO_2 and $[CO_2]$, but also velocities of reactions derived from pH dependent rate constants for hydration and dehydration of free CO_2 and HCO_3^- (Johnson 1982). The combination of pH dependent concentrations with pH dependent rate constants suggests that the uncertainties in the final derived reaction velocities could be greater than acknowledged by physiological studies which utilize these velocities (e.g. Burns & Beardall 1987, Merrett 1991, Colman & Rotatore 1995, Israel & González 1996, Korb et al. 1997, Laws et al. 1997).

It should be emphasized that the problems of 'between study' imprecision and inaccuracy by no means necessarily invalidate the conclusions of studies which have calculated pCO_2 and $[CO_2]$ using pH_{NBS} as a measurable. However, problems will materialize when comparisons are made between studies of calculated concentrations, or when critical 'threshold' concentrations or partial pressures derived in the laboratory are extrapolated to the ocean. The variation in estimated pCO_2 from a single pH_{NBS} & A_T pair is around 120 ppmv (Figs. 2 & 3) at atmospheric equilibrium of 360 ppmv, which is almost of the order of the variation in pCO_2 (around 160 ppmv) between glacial periods and the present day (Barnola et al. 1987). Clearly, in its present form, the pH_{NBS} & A_T calculation lacks the resolution required to address the biogeochemical significance of key physiological processes in marine phytoplankton, and how these might have varied with changing oceanic conditions over geological time.

It has been suggested that it is twice as accurate to directly measure pCO_2 as to calculate it (Dickson & Riley 1978). This is probably true using the highest precision IRGA instruments currently available. Our measurements were limited in precision (about 10 ppmv) by the resolution of the analogue instrument employed, and in accuracy by this resolution and by the lack of a correction for atmospheric pressure to convert ppmv to µatm (error of about ± 5 to 10 µatm). Thus, estimated accuracy of our measured pCO_2 was of the same order as that of our calculated values, which had a total analytical uncertainty (ignoring f_H problems) of about 3 to 4% (~10 to 15 ppmv at 360 ppmv; Fig. 2, Table 1). When the errors regarding equilibrium constants and f_H were also considered (Figs. 2 & 3), total uncertainty of calculated pCO_2 was around an order of magnitude greater (around 120 ppmv at 360 ppmv) and so calculation of pCO_2 or $[CO_2]$ from pH_{NBS} as a measurable can be recommended no longer without consideration of f_H.

Highly accurate and precise methods for analysis of the CO_2 equilibrium in the oceans are now available, methods such as spectrophotometric and potentiometric pH analyses (Byrne & Breland 1989, Dickson & Goyet 1994), potentiometric titration of A_T (Millero et al. 1993b, DOE 1994), and coulometric determination of C_T (e.g. Johnson et al. 1993, DOE 1994) and pCO_2 using gas chromatography or infra-red gas analysis (Wanninkhof & Thoning 1993, DOE 1994). However, many physiological studies do not have access to the equipment required for such analyses, which also need considerable technical and theoretical expertise. Moreover, relatively large sample volumes are required, which can compromise studies using small volume cultures in artificial seawater. Changes in the CO_2 equilibrium in nutrient supplemented cultures tend to be relatively large, and so methods of the highest precision and accuracy may not be as necessary as for oceanic measurements. One of the aims of this study was to determine whether a working value for f_H could be recommended so that a modification of the relatively simple pH_{NBS} & A_T method could be retained for use in culture studies, but this is clearly impossible given the electrode specificity of f_H. However, if direct measurements of f_H are made, and the true accuracy of the single point A_T titration determined, then the continued use of the pH & A_T method is possible, and would clearly be desirable in terms of working in cultures of phytoplankton. Indeed the analytical precisions of triplicate pH_{NBS}, A_T and calculated pCO_2 were excellent (Table 1); estimated inaccuracy of calculated pCO_2 resulted from the choice and misuse of equilibrium constants, and from invalid assumptions regarding $pH = -\log[H^+]$.

Clearly, there could now be much confusion on how best to approach the calculation of the CO_2 equilibrium in cultures of marine phytoplankton, and more work will be needed on this question before a clear strategy can be suggested. In the meantime we propose a number of interim recommendations:

(1) pCO_2 should preferably be measured directly, and if $[CO_2]$ is required then this can be calculated from pCO_2 and the solubility coefficient K_0. Direct measurement of pCO_2 together with A_T or C_T would allow calculation of the equilibrium without the problems inherent to measurement of pH_{NBS}. However, some caution will be required under the low pCO_2 con-

ditions potentially encountered during the late stage of cultures (~10 ppmv, D.W.C. pers. obs.). Here pCO_2 is of a similar order both to the resolution of measurements, and to the corrections for water vapour and atmospheric pressure; under these conditions, analytical accuracy is clearly of paramount importance.

(2) Any use of pH_{NBS} as a measurable in calculations of the CO_2 equilibrium must take into consideration f_H, and this should be independently measured. For example, f_H can be determined from changes in pH upon additions of acid beyond the equivalence point in the titration of A_T (e.g. Culberson et al. 1970, Mehrbach et al. 1973, Perez & Fraga 1987).

(3) An alternative to the use of pH_{NBS} is to calibrate electrodes directly using 'total hydrogen ion scale' seawater buffers (see Dickson 1993b, Millero et al. 1993a, DOE 1994). This avoids consideration of f_H and allows direct use of pH_{TOT} measurements with the equilibrium constants derived on that scale (DOE 1994). However, it must be accepted that systematic uncertainties may still remain even in these constants (see footnotes to Table 1).

(4) If single point titration of A_T is to be used in combination with either of the above pH measurements, then it must be accurately measured, preferably by calibration against A_T standards now available. An advantage of using A_T is that the measurement is very simple, and samples do not have to be protected from the atmosphere prior to titration; exchange of free CO_2 does not influence A_T. One disadvantage of using A_T is that there are many acid-base contributions to A_T, such as boric, phosphoric and silicic acids, and these must be corrected for to give A_C which is used in CO_2 equilibrium calculations (see 'Materials and methods'). If artificial buffers are added in excess to the seawater, as in some recipes, then the A_T measurement cannot be used, as the artificial buffer becomes the major contributor to A_T and thus would require a major correction to give A_C. Direct measurement of C_T is an alternative option in combination with either of the above pH determinations; C_T is directly input into calculations thus avoiding the above complications with A_T.

Whatever approach is adopted, it is clear that major improvements in precision and accuracy are required in this field, and that these will only be realised through improved communication and collaboration between the fields of marine chemistry and phytoplankton physiology.

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LITERATURE CITED

Anderson DH, Robinson RJ (1946) Rapid electrometric determination of the alkalinity of sea water. Ind Eng Chem, Anal Ed 18:767–769

Barnola JM, Raynaud D, Korotkevich YS, Lorius C (1987) Vostok ice core provides 160,000-year record of atmospheric CO_2. Nature 329:408–414

Bates RG (1963) Determination of pH. Theory and practice. Wiley, New York

Brewer PG, Goldman JC (1976) Alkalinity changes generated by phytoplankton growth. Limnol Oceanogr 21:108–117

Burns BD, Beardall J (1987) Utilization of inorganic carbon by marine microalgae. J Exp Mar Biol Ecol 107:75–86

Butler RA, Covington AK, Whitfield M (1985) The determination of pH in estuarine waters. II: Practical considerations. Oceanol Acta 8:433–439

Byrne RH, Breland JA (1989) High precision multiwavelength pH determinations in seawater using cresol red. Deep Sea Res 36:803–810

Colman B, Rotatore C (1995) Photosynthetic inorganic carbon uptake and accumulation in two marine diatoms. Plant Cell Environ 18:919–924

Copin-Montegut C (1988) A new formula for the effect of temperature on the partial pressure of CO_2 in seawater Mar Chem 25:29–37

Culberson C, Pytkowicz RM, Hawley JE (1970) Seawater alkalinity determination by the pH method. J Mar Res 28:15–21

Dickson AG (1984) pH scales and proton-transfer reactions in saline media such as sea water. Geochim Cosmochim Acta 48:2299–2308

Dickson AG (1990) Thermodynamics of the dissociation of boric acid in synthetic sea water from 273.15 to 298.15K. Deep Sea Res 37:755–766

Dickson AG (1993a) The measurement of sea water pH. Mar Chem 44:131–142

Dickson AG (1993b) pH buffers for sea water media based on the total hydrogen ion concentration scale. Deep Sea Res 40:107–118

Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Res 34:1733–1743

Dickson AG, Riley JP (1978) The effect of analytical error on the evaluation of the components of the aquatic carbon-dioxide system. Mar Chem 6:77–85

DOE (1994) Handbook of methods for the analysis of the various parameters of the carbon dioxide system in sea water; version 2. Dickson AG, Goyet C (eds) ORNL/CDIAC-74. US Department of Energy

Dong LF, Nimer NA, Okus E, Merrett MJ (1993) Dissolved inorganic carbon utilization in relation to calcite production in *Emiliania huxleyi* (Lohmann) Kamptner. New Phytol 123:679–684

Edmond JM, Gieskes JMTM (1970) On the calculation of the degree of saturation of sea water with respect to calcium carbonate under in-situ conditions. Geochim Cosmochim Acta 34:1261–1291

Freeman KH, Hayes JH (1992) Fractionation of carbon isotopes by phytoplankton and estimates of ancient CO_2 levels. Global Biogeochem Cycles 6:185–198

Fry B (1996) $^{13}C/^{12}C$ fractionation by marine diatoms. Mar Ecol Prog Ser 134:283–294

Gieskes JM (1969) Effect of temperature on the pH of seawater. Limnol Oceanogr 14:679–685

Goyet C, Poisson A (1989) New determination of carbonic acid dissociation constants in seawater as a function of

temperature and salinity. Deep Sea Res 36:635–1654
Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of seawater analysis, 2nd edn. Verlag Chemie, Basel
Hansson I (1973a) A new set of pH-scales and standard buffers for sea water. Deep Sea Res 20:479–491
Hansson I (1973b) A new set of acidity constants for carbonic acid and boric acid in sea water. Deep Sea Res 20:461–478
Harrison PJ, RE Waters, FJR Taylor (1980) A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. J Phycol 16:28–35
Israel AA, González EL (1996) Photosynthesis and inorganic carbon utilization in *Pleurochrysis* sp. (Haptophyta), a coccolithophorid alga. Mar Ecol Prog Ser 137:243–250
Johnson KM, Wills KD, Butler DB, Johnson WK, Wong CS (1993) Coulometric total carbon dioxide analysis for marine studies: maximizing the performance of an automated continuous gas extraction system and coulometric detector. Mar Chem 44:167–187
Johnson KS (1982) Carbon dioxide hydration and dehydration kinetics in seawater. Limnol Oceanogr 27:849–855
Korb RE, Saville PJ, Johnston AM, Raven JA (1997) Sources of inorganic carbon for photosynthesis by three species of marine diatom. J Phycol 33:433–440
Laws EA, Popp BN, Bidigare RR, Kennicutt MC, Macko SA (1995) Dependence of phytoplankton stable isotope composition on growth rate and $[CO_2]_{aq}$: theoretical considerations and experimental results. Geochim Cosmochim Acta 59:1131–1138
Laws EA, Thompson PA, Popp BN, Bidigare RR (1997) Sources of inorganic carbon for marine microalgal photosynthesis: a reassessment of $\delta^{13}C$ data from batch culture studies of *Thalassiosira pseudonana* and *Emiliania huxleyi*. Limnol Oceanogr (in press)
Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18:897–907
Merrett MJ (1991) Inorganic carbon transport in some marine microalgal species. Can J Bot 69:1032–1039
Merrett MJ, Nimer NA, Dong LF (1996) The utilization of bicarbonate ions by the marine microalga *Nannochloropsis oculata* (Droop) Hibberd. Plant Cell Environ 19: 478–484
Millero FJ, Poisson A (1981) International one-atmosphere equation of state for sea water. Deep Sea Res 28:625–629
Millero FJ, Zhang JZ, Fiol S, Sotolongo S, Roy RN, Lee K, Mane S (1993a) The use of buffers to measure the pH of seawater. Mar Chem 44:143–152
Millero FJ, Zhang JZ, Lee K, Campbell DM (1993b) Titration alkalinity of seawater. Mar Chem 44:153–165
Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford
Perez FF, Fraga F (1987) The pH measurements in seawater on the NBS scale. Mar Chem 21:315–327
Purdie DA, Finch MS (1994) Impact of a coccolithophorid on dissolved carbon-dioxide in seawater enclosures in a Norwegian fjord. Sarsia 79:379–387
Rau GH, Riebesell U, Wolf-Gladrow D (1996) A model of photosynthetic ^{13}C fractionation by marine phytoplankton based on diffusive molecular CO_2 uptake. Mar Ecol Prog Ser 133:275–285
Rau GH, Takahashi T, Des Marais DJ (1989) Latitudinal variations in plankton $\delta^{13}C$: implications for CO_2 and productivity in past oceans. Nature 341:516–518
Raven JA (1993) Limits on growth rates. Nature 361:209–210
Raven JA, Johnston AM (1991) Mechanisms of inorganic-carbon acquisition in marine phytoplankton and their implications for the use of other resources. Limnol Oceanogr 36:1701–1714
Riebesell U, Wolf-Gladrow DA, Smetacek V (1993) Carbon dioxide limitation of marine phytoplankton growth rates. Nature 361:249–251
Riley JP, Chester R (1971) Introduction to marine chemistry. Academic Press, London, p 121–151
Robertson JE, Robinson C, Turner DR, Holligan P, Watson AJ, Boyd P, Fernández E, Finch M (1994) The impact of a coccolithophore bloom on oceanic carbon uptake in the northeast Atlantic during summer 1991. Deep Sea Res 41:297–314
Rotatore C, Colman B, Kuzma M (1995) The active uptake of carbon dioxide by the marine diatoms *Phaeodactylum tricornutum* and *Cyclotella* sp. Plant Cell Environ 18: 913–918
Roy RN, Roy LN, Lawson M, Vogel KM, Moore CP, Davis W, Millero FJ (1993b) Thermodynamics of the dissociation of boric acid in seawater at S = 35 from 0 to 55°C. Mar Chem 44:243–248
Roy RN, Roy LN, Vogel KM, Moore CP, Pearson T, Good CE, Millero FJ, Campbell DJ (1993a) Determination of the ionization constants of carbonic acid in seawater. Mar Chem 44:249–268
Skirrow G (1975) The dissolved gases — carbon-dioxide. In: Riley JP, Skirrow G (eds) Chemical oceanography, Vol 2, 2nd edn. Academic Press, London, p 1–92
Spencer CP (1965) The carbon dioxide system in seawater: a critical appraisal. Oceanogr Mar Biol Annu Rev 3 :31–57
Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis, 2nd edn. Bull Fish Res Bd Can 167
Thompson PA, Calvert SE (1994) Carbon-isotope fractionation by a marine diatom: the influence of irradiance, daylength, pH, and nitrogen source. Limnol Oceanogr 39:1835–1844
Uppström LR (1974) Boron/chlorinity ratio of deep sea water from the Pacific Ocean. Deep Sea Res 21:161–162
Wanninkhof R, Thoning K (1993) Measurement of fugacity of CO_2 in surface water using continuous and discrete methods. Mar Chem 44:198–204
Weiss RF (1974) Carbon dioxide in water and seawater — the solubility of a non-ideal gas. Mar Chem 2:203–215
Whitfield M, Butler RA, Covington AK (1985) The determination of pH in estuarine waters. I. Definitions of pH scales and the selection of buffers. Oceanol Acta 8:423–432

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