

## The Bunsen gas solubility coefficient of ethylene as a function of temperature and salinity and its importance for nitrogen fixation assays

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### Abstract

The acetylene reduction assay is a common method for assessing nitrogen fixation in a variety of marine and freshwater systems. The method measures ethylene, the product of the conversion of the gas acetylene to its reduced form by nitrogenase. Knowledge of the solubility of ethylene in aqueous solution is crucial to the calculation of nitrogen fixation rates and depends on the temperature and salinity of the assay conditions. Despite the increasing interest in marine nitrogen fixation, no gas solubility (Bunsen) coefficients for ethylene in seawater are published to date. Here, we provide a set of equations and present semiempirically derived Bunsen coefficients for ethylene in water (ranging from 0.069 to 0.226) for a range of temperatures and salinities that are relevant for aquatic nitrogen fixation. We apply these data to nitrogen fixation scenarios at different temperatures and salinities and stress the importance of using accurate Bunsen coefficients in nitrogen fixation assays.

The global nitrogen cycle is balanced by two opposing biochemical pathways: denitrification, which releases N<sub>2</sub> to the atmosphere, and biological nitrogen fixation, which fixes N<sub>2</sub> gas. In the ocean, a change in the rate of either of these pathways will result in a change in the global oceanic nitrogen inventory. Estimates of total global and marine nitrogen fixation increased from 90 to 250–500 and 14 to 80–200 × 10<sup>6</sup> tons of nitrogen year<sup>-1</sup>, respectively, over the past 30 y (Capone and Carpenter 1999). Karl et al. (2002), in a recent review on marine nitrogen fixation processes and their importance for the biogeochemistry of the oceans, state that these estimates of marine nitrogen fixation are subject to large uncertainties. It is therefore of wide scientific interest to verify and improve generally applied methods to measure nitrogen fixation.

Most commonly, nitrogen fixation is measured using the acetylene reduction assay (ARA). The acetylene reduction assay can be applied to various types of marine and freshwater environments such as water column studies, sediment incubations, seagrass meadows, and so on. This assay measures the

production of ethylene (C<sub>2</sub>H<sub>4</sub>) in a sample after incubation with acetylene (C<sub>2</sub>H<sub>2</sub>), which is an alternative substrate for the N<sub>2</sub> reducing enzyme nitrogenase. Ethylene is easily detected with high sensitivity using gas chromatography (Capone 1993; Capone and Montoya 2001; Montoya et al. 1996; Stewart et al. 1968). The solubility of C<sub>2</sub>H<sub>4</sub>, required for the calculation of N<sub>2</sub> fixation, is dependent on the temperature and salinity at which the assay is carried out.

The amount of C<sub>2</sub>H<sub>4</sub> dissolved in an aqueous phase can be calculated based on the amount of C<sub>2</sub>H<sub>4</sub> detected in the overlying gas phase by applying the Bunsen gas solubility coefficient. The Bunsen coefficient ( $\alpha$ ) represents the solubility of a real gas, where  $\alpha$  is the unit volume of gas ( $v_0$ ), reduced to  $T_0 = 273.15$  K and  $p_0 = 1$  atm (101.325 kPa), which is absorbed by unit volume ( $V$ ) of solvent at the temperature of the measurement when the partial pressure of the gas is equal to one standard atmosphere ( $p_0$ )

$$\alpha = \frac{v_0}{V} \quad (1)$$

Therefore, the amount of gas present in the aqueous phase equals

$$n_{aq} = \frac{p_{gT} \times \alpha \times V_{aq}}{R \times T_0} \quad (2)$$

where  $n_{aq}$  denotes the total amount of gas (mol) dissolved in the aqueous phase,  $p_{gT}$  is the partial pressure of the gas in the headspace (atm) at the temperature of measurement,  $\alpha$  is the Bunsen coefficient at the temperature and salinity of the

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measurement,  $V_{aq}$  is the volume of the aqueous phase (liter), and  $R$  is the gas constant (0.08206 atm L mol<sup>-1</sup> K<sup>-1</sup>).

The application of the Bunsen coefficient has been common practice throughout nitrogen fixation studies applying ARA (Flett 1976; Capone 1993; Capone and Montoya 2001). As with any analysis of dissolved gases in liquids, the accuracy of the measurement largely depends on the correct gas solubility coefficients. Flett et al. (1976) discussed the temperature effect on the solubility of ethylene in freshwater and stated that  $\alpha = 0.122$  at 20°C and 1 atm. To the best of our knowledge no published values of ethylene Bunsen coefficients as functions of temperature and salinity are available. Therefore, deriving and supplying appropriate Bunsen coefficients for nitrogen fixation studies in aquatic systems is necessary.

In the present paper we derive Bunsen coefficients appropriate for a variety of marine systems ranging in temperature from 0°C to 35°C and salinities from 0 to 40. Our calculations can be readily applied to determine Bunsen coefficients for any temperature and salinity. The example calculations demonstrate that significant errors can be generated in ARA dependent nitrogen fixation rates when the effects of salinity and temperature on ethylene solubility are not taken into account.

### Materials and procedures

All temperatures in this section are referred to as absolute temperature in Kelvin. Experimental data for the solubility of ethylene in water and aqueous electrolyte solutions are published by Clever et al. (1970), Grollman (1929), McAuliffe (1966), Morrison and Billett (1952), Narasimhan et al. (1981), Onda et al. (1970a, 1970b), Orcutt and SeEVERS (1937), Taft et al. (1955), Truchard et al. (1961), Wu et al. (1985), Yano et al. (1968), and Yano et al. (1974). The aforementioned publications are compiled and evaluated in the IUPAC Solubility Data Series by Hayduk et al. (1994). Recently, R. Battino (pers. comm. unref.) published gas solubility measurements for ethylene in water in Clever and Battino (2003). Additionally, technical solubility coefficients ( $\lambda$ ), Ostwald coefficients ( $\beta$ ), and Bunsen coefficients ( $\alpha$ ) of ethylene in water as a function of temperature are summarized in D'Ans et al. (1967), Dean (1999), and Wilhelm et al. (1977).

The technical solubility coefficient is defined as the volume of gas in cm<sup>3</sup> at 273.15 K and 1 at (technical atmosphere, 1 at = 0.9678 atm) partial pressure that is absorbed by 1 g of solvent:

$$\lambda = \frac{\alpha}{D} \times 0.9678 \quad (3)$$

where  $D$  is the density of the solvent (in g cm<sup>-3</sup>) at the temperature of the measurement. Additionally, Ostwald coefficients ( $\beta$ ) for ethylene in water can be converted into Bunsen coefficients as

$$\beta = \alpha \times \frac{(T/K)}{273.15} \quad (4)$$

The Ostwald coefficient is defined as the solubility of a real gas in unit volume gas per unit volume of pure solvent at the tem-

perature of measurement, where the partial pressure in the gas phase is one standard atmosphere. Commonly, gas solubility is also expressed as mole fraction ( $x$ ), which can be converted to  $\alpha$  as follows:

$$\alpha = \frac{x \times R \times T_o}{p_o} \times \frac{D}{M} \quad (5)$$

where  $D$  equals the density of the solvent at the temperature of measurement and  $M$  represents the molecular weight of the solvent. Since Flett's (1976) original description of the ARA method,  $\alpha$  has become the standard parameter describing the C<sub>2</sub>H<sub>4</sub> solubility, and we have retained this convention. The available gas solubility coefficients from the literature, converted to  $\alpha$ , are summarized in Fig. 1. We used these data to calculate Bunsen coefficients at relevant temperatures and salinities for marine nitrogen fixation studies. Further, we applied these Bunsen coefficients to a theoretical scenario of measured nitrogenase activity via ARA at different temperatures and salinities.

*Semiempirical calculation of ethylene solubility coefficient ( $\alpha$ ) in seawater*—We adapted the equation for the temperature dependence of gas solubility at a constant salinity from Weiss (1970) and obtained a fit of  $\alpha$  versus temperature (Fig. 1) based on the measurements by Clever et al. (1970), Grollman (1929), McAuliffe (1966), Morrison and Billett (1952), Onda et al. (1970a), Orcutt and SeEVERS (1937), R. Battino (pers. comm. unref.), Taft et al. (1955), Truchard et al. (1961), Wu et al. (1985), and Yano et al. (1968). Ethylene gas solubility coefficients published by D'Ans et al. (1967), Dean (1999), and Wilhelm et al. (1977) agree well with the data used but are either derived from Morrison and Billett (1952) or have undefined primary sources and therefore were not used in the calculations.

$$\ln \alpha_o = a_1 + \frac{a_2}{(T/K)} + a_3 \ln(T/K) \quad (6)$$

$$a_1 = -189.757$$

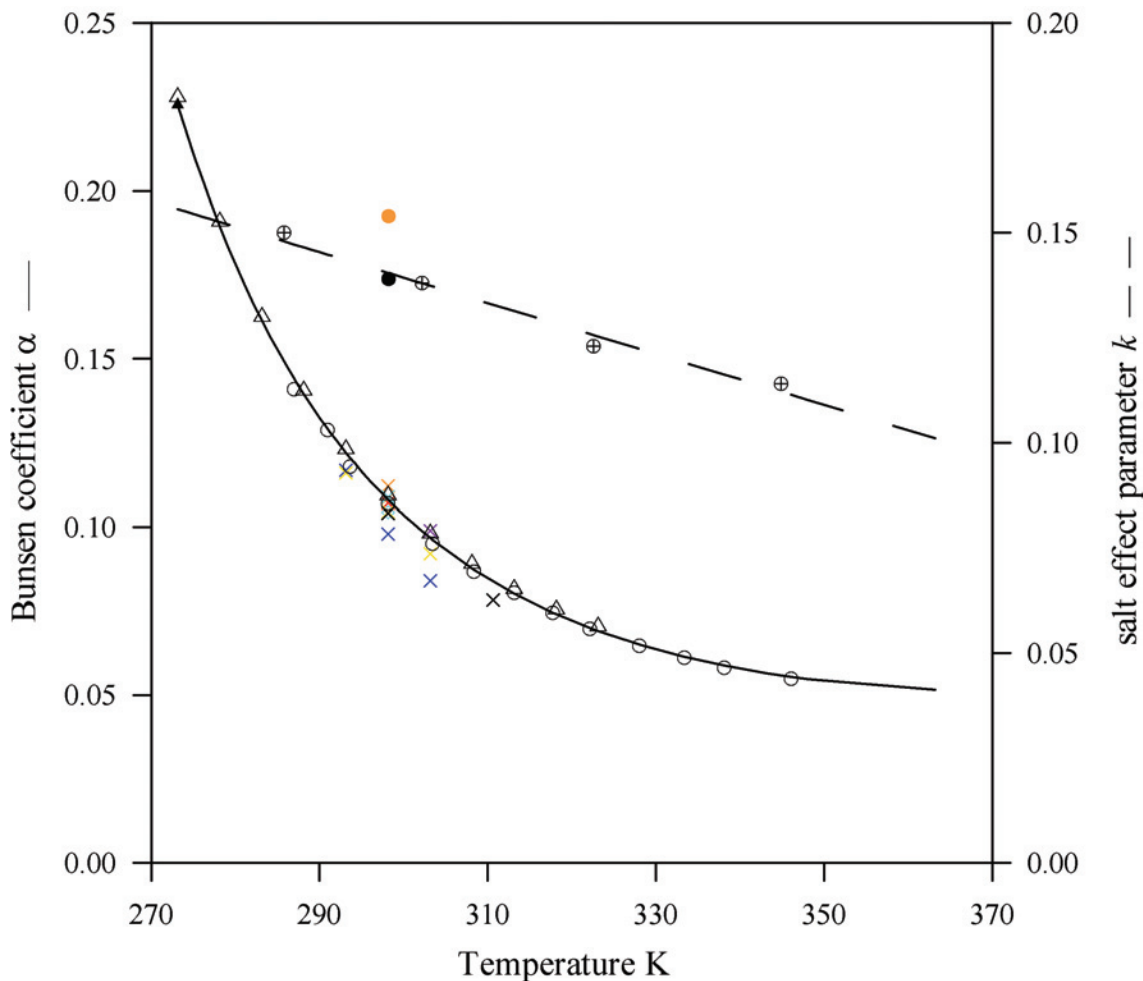
$$a_2 = 10092.7$$

$$a_3 = 26.9729$$

In addition to the temperature, the salt content influences the solubility of gases in liquids. The logarithm of the gas solubility in freshwater ( $\alpha_o$ ) over the gas solubility at a specific salt concentration ( $\alpha_i$ ) as a function of the ionic strength of the solution yields a linear dependency, where the slope ( $k$ ) is the empirical salt coefficient and can be used to correct  $\alpha$  relative to different ionic strengths  $I$  (Setschenov 1889):

$$\log_{10}(\alpha_o / \alpha_i) = k \times I \quad (7)$$

The ionic strength of a solution is defined as  $I = 0.5 \sum_i (c_i/c^o) z_i^2$ , where  $c_i$  is the molar concentration and  $z_i$  is the charge of the  $i$ th ion. The salt effect coefficient itself is temperature dependent.



**Fig. 1.** Temperature dependence of the Bunsen coefficient  $\alpha$  (for freshwater) and of the salt effect coefficient  $k$  (for NaCl). The temperature dependence is largely described by Morrison and Billett (1952) –  $\circ$  [black] and R. Battino (pers. comm. unref.) –  $\triangle$  [black]. Further measurements accounted for were published by Clever et al. (1970) –  $\times$  [purple], Grollman (1929) –  $\times$  [black], McAuliffe (1966) –  $\diamond$  [cyan], Narasimhan et al. (1981) –  $\times$  [blue], Orcutt and Seevers (1937) –  $\times$  [cyan], Taft et al. (1955) –  $\times$  [yellow], Truchard et al. (1961) –  $\blacktriangle$  [black], Wu et al. (1985) –  $\times$  [red] and Yano et al. (1968) –  $\times$  [orange]. The solid line represents the fit of the experimental data based on equation 6. Data for the salt effect coefficient ( $k$ ) are based on measurements of Morrison and Billett (1952) –  $\oplus$  [black], Onda et al. (1970a) –  $\bullet$  [black] and Yano et al. (1974) –  $\bullet$  [orange] and the dashed line represents a linear fit of  $k$  based on equation 8.

We derived a temperature function for  $k$  based on data for NaCl given by Morrison and Billett (1952) and Onda et al. (1970a). A linear function was found to be sufficient, since the application of a quadratic function had an insignificant effect on the calculated Bunsen coefficients as a function of temperature and salinity (Fig. 1):

$$k = k_1 + k_2 \times (T / K). \quad (8)$$

$$k_1 = 0.3212$$

$$k_2 = -6.063 \times 10^{-4}$$

The ionic strength of seawater at the desired salinity can be derived from the molar concentration of  $Cl^-$  ions ( $0.54588 \text{ mol L}^{-1}$ ) in seawater at a salinity ( $S$ ) of 35:

$$I = 1.278 \times [Cl^-] / \text{mol L}^{-1}$$

or

$$I = 0.01993 \times S. \quad (9)$$

Thus one can derive Bunsen coefficients as a function of temperature and salinity (Table 1) based on

$$\alpha = \exp \left[ \left( a_1 + \frac{a_2}{(T / K)} + a_3 \ln(T / K) \right) - 2.303 \times k \times I \right]. \quad (10)$$

#### Assessment

We derived semiempirical Bunsen coefficients for a range of seawater salinities and temperatures based on laboratory data obtained for freshwater of different temperatures and for NaCl solutions of different concentrations and temperatures. Of the

**Table 1.** Bunsen coefficients ( $\alpha$ ) for ethylene in water of different temperatures and salinities (S) at 1 atm

Temperature (°C)	S = 0	S = 5	S = 10	S = 15	S = 20	S = 25	S = 30	S = 32	S = 35	S = 40
0	0.226	0.218	0.210	0.203	0.196	0.189	0.182	0.180	0.176	0.170
2	0.210	0.203	0.196	0.189	0.182	0.176	0.170	0.167	0.164	0.158
4	0.196	0.189	0.183	0.176	0.170	0.164	0.159	0.156	0.153	0.148
5	0.189	0.183	0.177	0.171	0.165	0.159	0.154	0.151	0.148	0.143
6	0.183	0.177	0.171	0.165	0.159	0.154	0.149	0.147	0.144	0.139
8	0.172	0.166	0.160	0.155	0.150	0.144	0.140	0.138	0.135	0.130
10	0.161	0.156	0.151	0.146	0.141	0.136	0.131	0.130	0.127	0.123
12	0.152	0.147	0.142	0.137	0.133	0.128	0.124	0.122	0.120	0.116
14	0.143	0.139	0.134	0.130	0.125	0.121	0.117	0.116	0.113	0.109
15	0.139	0.135	0.130	0.126	0.122	0.118	0.114	0.112	0.110	0.107
16	0.136	0.131	0.127	0.123	0.119	0.115	0.111	0.109	0.107	0.104
18	0.128	0.124	0.120	0.116	0.113	0.109	0.105	0.104	0.102	0.099
20	0.122	0.118	0.114	0.111	0.107	0.103	0.100	0.099	0.097	0.094
22	0.116	0.112	0.109	0.105	0.102	0.099	0.095	0.094	0.092	0.089
24	0.111	0.107	0.104	0.100	0.097	0.094	0.091	0.090	0.088	0.085
25	0.108	0.105	0.101	0.098	0.095	0.092	0.089	0.088	0.086	0.084
26	0.106	0.102	0.099	0.096	0.093	0.090	0.087	0.086	0.084	0.082
28	0.101	0.098	0.095	0.092	0.089	0.086	0.083	0.082	0.081	0.078
30	0.097	0.094	0.091	0.088	0.085	0.083	0.080	0.079	0.078	0.075
32	0.093	0.090	0.087	0.085	0.082	0.080	0.077	0.076	0.075	0.072
34	0.089	0.087	0.084	0.081	0.079	0.077	0.074	0.073	0.072	0.070
35	0.088	0.085	0.082	0.080	0.078	0.075	0.073	0.072	0.071	0.069

major electrolytes in seawater (NaCl, MgCl<sub>2</sub>, Na<sub>2</sub>SO<sub>4</sub>, CaCl<sub>2</sub>, KCl, NaHCO<sub>3</sub>, KBr, and CsBr), Hayduk et al. (1994) summarize salt effect coefficients for NaCl, Na<sub>2</sub>SO<sub>4</sub>, KCl, NaHCO<sub>3</sub>, and KBr at 298.15 K. However, the salt effect coefficient as a function of temperature is only reported for NaCl (data from Morrison and Billett 1952). In our analysis we exclusively used NaCl-based data to derive the temperature- and salinity-dependent salt effect coefficient. The composition of seawater was taken into account by using the ionic strength of seawater as given by Eq. 8. Thus, we assumed that all single charged electrolytes such as NaCl ( $\sum z_i^2 = 2$ ) have a similar salt effect coefficient, whereas double charged electrolytes such as Na<sub>2</sub>SO<sub>4</sub> ( $\sum z_i^2 = 6$ ) have a three times higher  $k$  value. Actually, salt effect coefficients (in parentheses) of single charged electrolytes NaCl (0.139) (Onda et al. 1970a), KCl (0.136), and KBr (0.118) (Yano et al. 1974) are indeed in relatively close agreement with each other, whereas Na<sub>2</sub>SO<sub>4</sub> (0.394) is 2.8 times higher and thus lies within the expectation (Onda et al. 1970a). However for electrolytes such as NaHCO<sub>3</sub> (0.195), a simple ionic strength-based method seems less accurate (Onda et al. 1970b). Nevertheless, the mole fraction ( $x_i$ ) of NaCl in seawater relative to the total salt content is very high (0.837). Despite the lack of data for MgCl<sub>2</sub> ( $x_i = 0.104$ ) and the minor importance of KBr ( $x_i = 0.0015$ ) and NaHCO<sub>3</sub> ( $x_i = 0.005$ ), we are confident that the use of the salt effect coefficient of NaCl, in combination with the application of the ionic strength of seawater, is sufficient to account for the salting-out effect of seawater (Millero 1996;

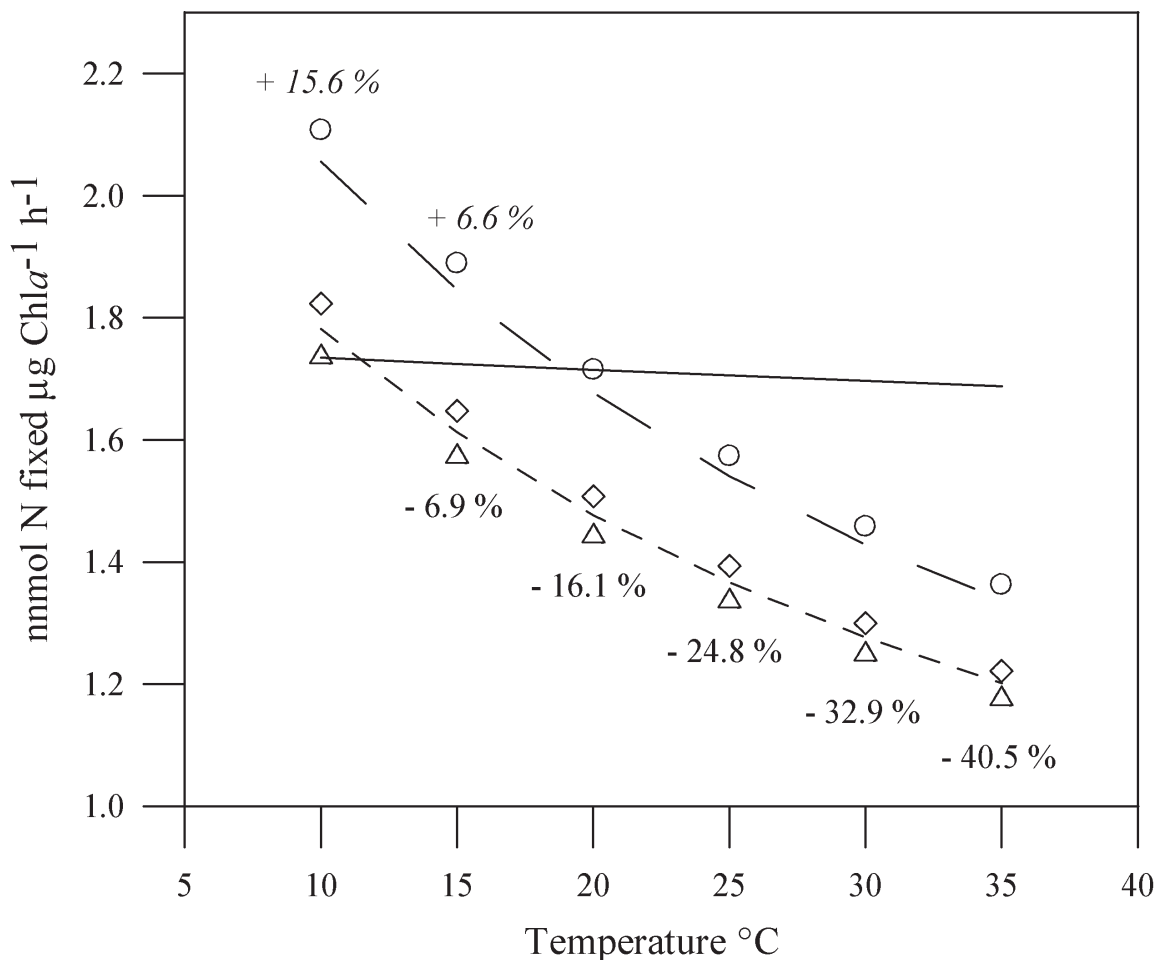
Schwarzenbach et al. 1993). To the best of our knowledge, there are no other data for the salting-out effect of NaCl than those given by Morrison and Billett (1952), Onda et al. (1970a), Yano et al. (1974) (0.154), and the measurements published in D'Ans et al. (1967) (0.140) available. Based on the recommendation by Hayduk et al. (1994), preference was given to the measurements by Morrison and Billett (1952) and Onda et al. (1970a). The semiempirical approach presented here yields the most representative Bunsen coefficients for ethylene in seawater to date. Direct measurements of ethylene solubility in natural seawater are needed to verify or further improve these calculations.

The practical details of the ARA are well described (Capone 1993; Capone and Montoya 2001). In general the acetylene reduced to ethylene during ARA is detected using flame ionization detection–gas chromatography. In the following, an ARA case study serves as an illustrative example for the crucial role of the Bunsen coefficient of ethylene in nitrogen fixation studies.

In ARA, the total amount of ethylene produced in the assay vessel is determined by measuring the partial pressure of ethylene in the headspace. The amount of ethylene present in the gas phase is then given by the ideal gas law:

$$n_g = \frac{p_{gT} \times V_g}{R \times T} \quad (11)$$

where  $n_g$  is the amount of ethylene in mol,  $p_{gT}$  equals its partial pressure (atm) at the temperature of measurement  $T$  (K),  $V_g$  is



**Fig. 2.** Theoretical nitrogen fixation rates at temperatures and salinities relevant for marine nitrogen fixation using semiempirically derived Bunsen coefficients.  $\circ$  represents values at  $S = 0$ ,  $\diamond$  at  $S = 30$ , and  $\triangle$  at  $S = 40$ . The solid line represents theoretical nitrogen fixation rates using a Bunsen coefficient of 0.122 independent of  $T$  and  $S$  in comparison with fixation rates based on  $T$  and  $S$  dependent Bunsen coefficients as derived in this work (---). Percentages illustrate difference between nitrogen fixation rates at a salinity of 35 (---) and 5 (— — —, values in *italics*) versus  $\alpha = 0.122$ .

the headspace volume in liters,  $R$  equals the gas constant =  $0.08206 \text{ atm L mol}^{-1} \text{ K}^{-1}$ . Because the amount of  $C_2H_4$  present in the aqueous phase can be calculated based on Eq. 2, the total amount of  $C_2H_4$  produced in the assay equals the sum of Eq. 2 and Eq. 11.

These calculations were applied to a theoretical scenario of 85 ppm ethylene production detected in a gas sample from a 20.2-mL headspace vial generally used in our laboratory (19 mL culture and 1.2 mL headspace) over a range of temperatures and salinities. We assumed a biomass of  $50 \mu\text{g}$  chlorophyll  $a$  ( $\text{Chl } a$ )  $\text{L}^{-1}$  and an incubation time of 2 h. These values were based on common conditions and observations for ARA-dependent nitrogen fixation studies using cultures of the cyanobacteria *Trichodesmium* (strain IMS 101) in our laboratory. We calculated theoretical nitrogen fixation rates using a ratio of  $C_2H_2$  reduced :  $N_2$  reduced of 4:1 (Montoya et al. 1996) for temperatures between  $10^\circ\text{C}$  and  $35^\circ\text{C}$  and salinities ranging from 0 to 40 ppm (Fig. 2) using the Bunsen coefficients from Table 1. Additionally,

we compared these results to calculated nitrogen fixation rates based on identical settings, but using a Bunsen coefficient of 0.122 independent of temperature and salinity. This exercise illustrates how differences in  $\alpha$  result in significant differences in ARA determined nitrogen fixation rates.

With the exception of Flett (1976), Bunsen coefficients are generally not reported in studies of marine nitrogen fixation. Identical to Dean (1999) and in agreement with our calculations, Flett (1976) reports  $\alpha = 0.122$  as a value for freshwater at  $20^\circ\text{C}$  but gives no information on  $\alpha$  as a function of salinity and temperature. Our calculations show that Bunsen coefficients range from 0.069 to 0.226 for temperatures ( $0^\circ\text{C}$  to  $35^\circ\text{C}$ ) and salinities (0 to 40) relevant for aquatic nitrogen fixation studies (Table 1). As a function of temperature and salinity, nitrogen fixation rates range between 1.18 (salinity = 40, temperature =  $35^\circ\text{C}$ ) and 2.11  $\text{nmol N fixed } (\mu\text{g Chl } a)^{-1} \text{ h}^{-1}$  ( $S = 0$ ,  $T = 10^\circ\text{C}$ ). In contrast, nitrogen fixation rates only vary from 1.69 to 1.73  $\text{nmol N fixed } (\mu\text{g chl-}a)^{-1} \text{ h}^{-1}$  over a temperature range from

10°C to 35°C when a Bunsen coefficient of 0.122 is used (Fig. 2). Employing  $\alpha = 0.122$  in assays with a temperature higher than 19°C thus yields overestimation of nitrogen fixation rates regardless of the salinity and an underestimation in freshwater assays at temperatures lower than 19°C. If the ARA is conducted in oceanic environments ( $S \approx 35$ ), underestimation and overestimation of nitrogen fixation will occur if  $\alpha = 0.122$  is employed below or above 11.5°C, respectively (Fig. 2). Because gas solubility is negatively correlated with temperature and salinity, the strongest deviations from results that apply  $\alpha = 0.122$  arise at high temperatures and salinities ( $>40\%$  for  $T = 35$ ,  $S \geq 35$ ). These deviations are large compared to the precision of the gas phase analysis. Gas chromatography using flame ionization detection responds linearly to the concentration of  $C_2H_4$ , and a detection limit as low as 2.5 ppb can be achieved. Thus errors in a calibration curve generated by imprecise calibration standards are obvious and can be excluded. Note that the ratio of the volume in the aqueous phase to the volume of the headspace has an effect on the sensitivity of the method, and optimum volume ratios may need to be determined for each assay setup individually (Montoya et al. 1996).

### Discussion

Whereas high salinities and temperatures are of particular interest for studies of nitrogen fixation in vast regions of oligotrophic tropical and subtropical oceans, lower temperatures and brackish waters are encountered in temperate fjords and semi-enclosed oceans such as the Baltic Sea, which experience seasonal episodes of nitrogen fixation. Here, a salinity range of 5 to 15 and a water temperature of 10°C to 20°C are common in regions possessing cyanobacterial summer blooms. For example, blooms of *Nodularia* spp., *Anabaena* spp., and *Aphanizomenon* spp. regularly occur during late summer in the Bothnian Sea and the Gulf of Finland where salinity averages 5 to 6 and water temperature 10°C to 14°C. Similar blooms have also been reported from the Western Baltic where the water temperature ranges from 15°C to 20°C in the summer with predominant salinities around 15 (Gallon et al. 2002; Lozán et al. 1996). At such a wide range of temperature and salinity, the Bunsen coefficients corrected for temperature and salinity are important for the accuracy of the  $N_2$  fixation rate estimates. In some systems,  $\alpha$  can deviate as much as 28% from 0.122 ( $\alpha = 0.156$ ,  $T = 10^\circ\text{C}$ ,  $S = 5$ ) and nitrogen fixation rates can be underestimated by 15.6% if measurements employ  $\alpha = 0.122$ . If studies are carried out in freshwater systems, deviations can even be larger (Table 1, Fig. 2).

In the open ocean salinity shows much less considerable change ( $S \approx 35$ ) and temperatures regularly do not exceed 30°C (Levitus and Boyer 1994). The application of  $\alpha = 0.122$  deviates up to 36% from appropriate values ( $\alpha = 0.078$ ,  $T = 30^\circ\text{C}$ ,  $S = 35$ ) and results in a bias toward too high  $N_2$  fixation rate measurements by ~33% (Table 1, Fig. 2). The salt effect on the solubility of ethylene in seawater compared to the temperature effect can sometimes be negligible in oceanic systems. In

contrast,  $N_2$  fixation rates can be overestimated by more than 40% in tropical lagoons and other semienclosed water bodies when temperatures are elevated up to 35°C and the salinity is increased above 35 (Fig. 2).

The application of the ARA as a true measure for nitrogen fixation relies on the fact that nitrogenase is the only enzyme that reduces acetylene to ethylene and is based on a theoretical ratio between the rate of acetylene reduction to cellular  $N_2$  reduction of 4:1 (Postgate 1982; Montoya et al. 1996; Gallon et al. 2002). However, this ratio frequently deviates from its theoretical value. Gallon et al. (2002) demonstrates that even though  $^{15}N_2$  nitrogen fixation measurements accurately measure incorporation of nitrogen into cellular material, measurements of acetylene reduction more truly reflect the gross rate of  $N_2$  fixation. The  $^{15}N_2$  assay can underestimate the actual amount of  $N_2$  fixed, because the population under investigation can release a significant portion of its newly fixed nitrogen (Bronk 2002) stressing the importance of the ARA for field measurements of nitrogen fixation. In our view, it also supports the need for increased accuracy of this method as provided here.

### Comments and recommendations

Based on our calculations of Bunsen coefficients we agree with the validity of the recommendation by Flett et al. (1976), Capone (1993), and Capone and Montoya (2001) stating that appropriate gas solubility coefficients for ethylene must be applied for calculating nitrogen fixation rates and advise future authors to report the gas solubility coefficients used in their assays. Using available experimental data we provide interpolation formulas and also a convenient table (Table 1) for  $\alpha$  values at temperature/salinity combinations of interest in aquatic research. Further, we stress the need for measurements of gas solubility coefficients of ethylene in seawater under temperature and salinity conditions that are relevant to marine nitrogen fixation studies. Estimates of the oceanic nitrogen budget as well as biogeochemical models, largely rely on ARA results to calculate  $N_2$  fixation rates (Capone and Carpenter 1999; Fennel et al. 2001; Hood et al. 2001). The tropical/subtropical cyanobacterium *Trichodesmium* spp. or *Richelia* spp. have been assumed to be the major representatives of oceanic nitrogen fixers. Zehr et al. (2001) recently discovered unicellular diazotrophs, but their distribution as well as their contribution to the oceanic nitrogen budget is unknown. Thus measurements of marine nitrogen fixation may be extended to regions of different temperature and salinity than assessed to date. The need for standardized methods of nitrogen fixation measurements is apparent, and our contribution is intended to be a step toward that goal.

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