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The relationship between dissolved hydrogen and nitrogen fixation in ocean waters

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ABSTRACT

Fixed nitrogen is a key nutrient involved in regulating global marine productivity and hence the global oceanic carbon cycle. Oceanic nitrogen (N_2) fixation is estimated to supply 8×10^{12} moles N y^{-1} to the ocean, approximately equal to current riverine and the atmospheric inputs of fixed N, and between 50 and 100% of current estimates of oceanic denitrification. However, the spatial and temporal variability of N_2 fixation remains uncertain, mostly because of the normal low resolution sampling for diazotroph distribution and fixation rates. It is well established that N_2 fixation, mediated by the enzyme nitrogenase, is a source of hydrogen (H_2), but the extent to which it leads to supersaturation of H_2 in oceanic waters is unresolved. Here, we present simultaneous measurements of upper ocean dissolved H_2 concentration (nmol L^{-1}), and rates of N_2 fixation (μmol N m^{-3} d^{-1}), determined using $^{15}N_2$ tracer techniques (at 7 or 15 m), on a transect from Fiji to Hawaii. We find a significant correlation ($r = 0.98$) between dissolved H_2 and rates of N_2 fixation, with the greatest supersaturation of H_2 and highest rates of N_2 fixation being observed in the subtropical gyres at the southern (~18°S) and northern (18°N) reaches of the transect. The lowest H_2 saturation and N_2 fixation were observed in the equatorial region between 8°S and 14°N. We propose that an empirical relationship between H_2 supersaturations and N_2 fixation measurements could be used to guide sampling for ^{15}N fixation measurements or to aid the spatial interpolation of such measurements.

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1. Introduction

The existence of loss processes for combined nitrogen in the ocean, in particular denitrification, makes the counterbalancing process of biological N_2 fixation one of the most important in maintaining marine primary production. Following from this, N_2 fixation may over long time scales play a role in the distribution of CO_2 between the atmosphere and deep ocean (e.g. Falkowski, 1997). In spite of this central role and decades of study of the process, the magnitude and spatial and temporal variability of N_2 fixation remain inadequately determined. The identity of the organisms responsible and their mode of operation continue to be topics of intensive research (Church et al., 2005; Foster and Zehr, 2006). One of the difficulties of identifying and quantifying N_2 fixation is that few of its effects can be measured quickly and directly. The uptake of elemental nitrogen itself is not easily measured against the high background of dissolved N_2 which is caused by its relatively high solubility and high atmospheric abundance. Also, there are other processes causing deviations from saturation, such as bubble injection and changes in temperature and atmospheric pressure. Nitrogen fixation activity is now

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commonly quantified through $^{15}N_2$ incubations (Montoya et al., 1996) and subsequent analysis by isotope ratio mass spectrometry. Although sensitive and selective, this method is not normally found to be practical on board ship, so incubated samples are stored for analysis ashore. The traditional acetylene reduction technique (Stewart et al., 1967; Zehr and Montoya, 2007), which employs gas chromatography with a flame ionization detector (GC-FID), lacks the sensitivity to be easily used on cyanobacteria at normal ocean abundance, although recent method development may overcome these difficulties (Staal et al., 2007). However, it is possible that hydrogen (H_2) release during N_2 fixation could leave a measurable signal that would have some utility in the study of this process.

Hydrogen is a trace component of the atmosphere that is produced during the photolysis of atmospheric formaldehyde, itself an intermediate in the oxidation of methane initiated by reaction with OH (Simmonds et al., 2000). Other major sources are the burning of biomass and fossil fuels (Novelli et al., 1999). Uptake by soils is the predominant sink, accounting for about 80% of the loss from the atmosphere (Xiao et al., 2007). A second significant loss is through oxidation by atmospheric OH (Levy, 1972).

Hydrogen is typically supersaturated in low latitude surface waters with concentrations that decrease exponentially below the mixed layer (Herr et al., 1984; Scranton et al., 1982; Scranton, 1984; Conrad and Seiler, 1988). At higher latitudes supersaturations decrease, and a few high latitude surveys show undersaturations in surface waters (Herr et al., 1981, 1984; Punshon et al., 2007). It has long been recognized that there are two possible sources of surface H_2 supersaturation. One is photochemical production which was suggested to be responsible for an observed positive correlation between dissolved H_2 in surface waters and solar irradiance in a study in the South Atlantic (Herr et al., 1984). A recent study of photochemical production of H_2 in natural waters revealed that the effect was most pronounced in highly coloured lake waters, but occurred also in coastal water samples (Punshon and Moore, 2008b). No data were available for open ocean waters, but the study suggested that some production would be expected based on a mechanism involving photolysis of coloured dissolved organic matter (CDOM). The alternative, or complementary, source from biological nitrogen fixation is better understood, although not all details of the mechanism are known. It appears that the release of a H_2 molecule from dinitrogenase (already in a partially reduced state) occurs with the uptake of a N_2 molecule prior to its stepwise reduction to ammonia (Lowe and Thorneley, 1984; Postgate, 1998; Newton, 2007). The stoichiometry of nitrogen reduction is given by:

$$N_2 + 8H^+ + 16\ ATP + 8e^- \rightarrow 2NH_3 + H_2 + 16ADP$$

Thus one H_2 is released for each N_2 reduced. However, H_2 can be oxidised through the action of a hydrogenase and hence provide reducing power for the above reaction (Fisher and Newton, 2002). While it may be expected that H_2, as an energy source, would be taken up, either by the N_2-fixing organism itself, or by other members of the microbial community, a signal of supersaturation should appear in the water, provided that the release and uptake are not closely coupled. The level of supersaturation would depend on the rate of H_2 loss to the atmosphere, and hence on wind speed, and the depth of the mixed layer in which production is occurring as well as the strength of the microbial uptake sink. The magnitude of the supersaturation is enhanced by the sparing aqueous solubility of H_2; for example, the production of 1 nmol L^{-1} of NH_3 (a typical daily fixation rate in tropical waters), if matched by the stoichiometric release of 0.5 nmol L^{-1} of H_2, would yield a saturation level of 240% in water that was previously at equilibrium with the atmosphere at 25 °C.

Production of H_2 during nitrogen fixation by the marine diazotroph, *Trichodesmium*, has been demonstrated in laboratory experiments (Scranton, 1983; Punshon and Moore, 2008a) and it has been recognized as a probable source of H_2 supersaturation in tropical waters (Herr and Barger, 1978; Scranton, 1983). With H_2 production being a fundamental part of the mechanism of N_2 fixation by nitrogenases, it is to be expected that more recently discovered marine diazotrophs would act as a source of H_2 in the ocean, but it cannot be assumed that the ratio of its net release relative to N_2 fixed would be the same as for *Trichodesmium*. We are not aware of any studies that have been made of H_2 release by the unicellular cyanobacterium, *Crocosphaera* (which has the ability to fix N_2 at night). Most recently Zehr et al. (2009) have reported the existence of uncultivated unicellular marine diazotrophic cyanobacteria that do not fix CO_2 or release O_2 since they lack photosystem II. These organisms, free of inhibition of N_2-fixation by photosynthetic O_2, are also likely to play a role in the marine H_2 cycle.

In the current study, dissolved H_2 was measured in an ocean transect, for the first time paralleled by $^{15}N_2$ incubations to quantify N_2 fixation. The objectives of this study were to determine whether N_2 fixation yields a significant transient signal of H_2 supersaturation in surface ocean waters, and whether the signal may be correlated with the rate of N_2 fixation.

It is recognised that proceeding from any qualitative relationship to a quantitative one is likely to be challenging because the concentration of H_2 in seawater is dependent on the balance between a number of production and loss processes, rather than on a single production process such as N_2 fixation. The loss processes in surface waters will include loss to the atmosphere at a rate (per litre of water) that is dependent on wind speed, the degree of supersaturation, and the depth of the mixed layer (which determines the size of the exchangeable reservoir). In addition to this physical loss, there can be a biological loss, which would be a function of the species of H_2 consumers present, their abundance, and the state of their metabolism.

2. Methods

2.1. Hydrogen saturation

The work was done mainly during a transit of R/V Kilo Moana from Suva, Fiji to Honolulu, Hawaii in April 2007

on the C-MORE sponsored BULA cruise (Fig. 1); reference is also made to work done on the same vessel during a cruise to the Hawaii Ocean Times-Series (HOT) sampling site Station ALOHA (22° 45′N, 158°W), May 3–7, 2007. The technique for measuring H_2, involving its GC separation from other reducing gases, oxidation over heated HgO and detection by UV absorption is described in Punshon et al. (2007). In brief, seawater samples were drawn through a long Teflon^{TM} needle into a 50 mL glass syringe fitted with a 3-way nylon stopcock and the sample volume adjusted to 35 mL. A 5 mL headspace of zero air, free of H_2 and CO, was then introduced and the syringe shaken vigorously for 3 minutes. This time period had previously been shown to result in complete phase equilibration. The headspace was then transferred to the sample loop (1 mL) of a reduction gas analyser (Peak Laboratories, USA). Phase equilibration was conducted at ambient water temperature with the equilibration temperature for each sample being determined from measurements made immediately before and after equilibration. Calibration was effected using a 0.9 ppm primary standard prepared by gravimetric dilution of volumetrically measured H_2 with ultra high purity N_2 in a 4.5 L internally electro-polished stainless steel tank (Essex Cryogenics). Multi-point calibration curves were constructed daily by diluting volumes of the primary standard with H_2-free zero air in a glass air sampling syringe to produce H_2 mixing ratios in the range 0–0.9 ppm. The coefficient of variation of the analysis is $\pm$2% and the detection limit 20 pmol L^{-1}. The solubility of H_2 was calculated using the T and S dependent relationship of Wiesenburg and Guinasso (1979).

During the transect there was frequent sampling from the ship's supply of seawater which is delivered through a Teflon-lined stainless-steel pipe from a pump located in the forward starboard hull drawing in water from a depth of 7 m. Samples were normally taken from a seawater tap in an aft laboratory but several samples were also drawn from a tap adjacent to this pump. Additionally, samples were taken from 10 L Niskin bottles during 8 vertical profiles. To minimize potential contamination from the rosette (resulting from corrosion, e.g. of a zinc sacrificial anode), the bottles to be used for H_2 analysis were closed during the upward profile as soon as the bottle reached the intended sampling depth. (Potential sources of contamination and how they might be avoided have been discussed by Scranton et al., 1982.) Water was fed into the bottom of an oxygen bottle (300 mL) via a Tygon tube with the bottle being allowed to overflow by ~2 volumes before it was stoppered. A few samples were taken with a plastic bucket and rope from the side of the ship with efforts made to avoid water aerated by propeller wash. Duplicate samples were immediately drawn from the bucket into 50 ml glass syringes. These samples, together with those taken near the inlet to the ship's supply, are

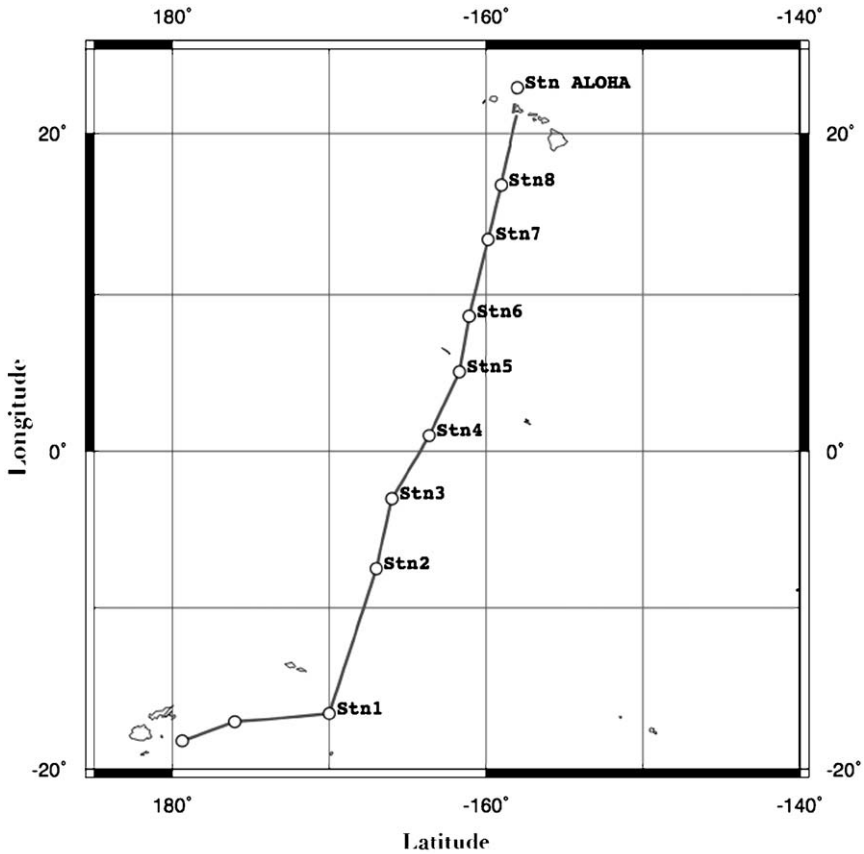


Fig. 1. Cruise track of Kilo Moana showing station locations and the position of the Hawaii Ocean Time Series station.

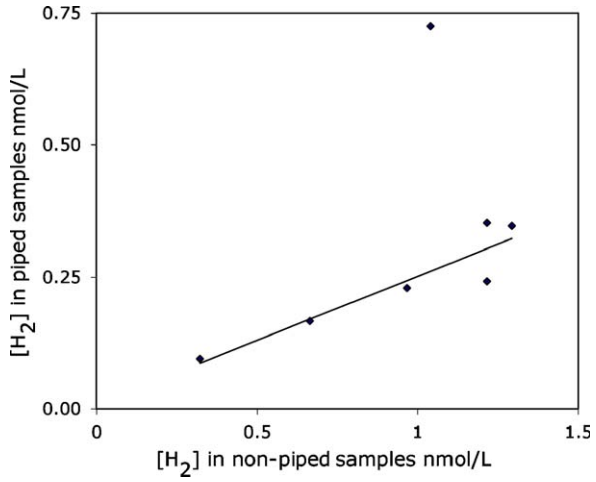


Fig. 2. Plot of H_2 in water from ship's uncontaminated supply ("piped" samples) plotted against values for samples that did not pass through the ship's plumbing. The regression line ($y = 0.009+0.243x$; $r^2 = 0.86$) excludes the single outlying data point.

referred to as "non-piped" to distinguish them from the laboratory tap samples that had passed through the ship's plumbing ("piped") (Fig. 2). Dissolved H_2 was measured typically within 1.5 hours.

The criteria for determining mixed layer depths were a rate of change in salinity and temperature of $0.002\,m^{-1}$ and $0.01\,^{\circ}C\,m^{-1}$ respectively. Samples for determination of nitrate and nitrite concentrations were collected from CTD stations at ten depths between 0 and 1000 m. To determine surface ocean, low level nitrate plus nitrite concentrations ($1\,nmol\,kg^{-1}$ to $1\,\mu mol\,kg^{-1}$), chemiluminescence techniques were employed using an Antek NO_x analyser (Garside, 1982). For samples containing nitrate plus nitrite at concentrations $>1\,\mu mol\,kg^{-1}$, standard continuous flow auto-analytical techniques were employed using a Bran and Luebbe AA3 analyzer (Technicon, 1977). High-resolution temperature data were collected from the ship's flow-through seawater system using a SeaBird SBE-21 Seacat thermosalinograph. Samples for measurement of chlorophyll-a concentrations ($\mu g\,L^{-1}$) were collected at CTD and underway stations. Typically, 100 mL of water was collected and filtered immediately through a 25 mm glass fiber filter (GF/F, 0.7 μm). The filter was stored and chlorophyll-a extracted in 100% acetone at $-20\,^{\circ}C$ and concentrations determined using a Turner 10AU fluorimeter (Strickland and Parsons, 1972). Exchange velocities used in the calculation of the flux of H_2 between the ocean and atmosphere were determined from the relationship of Wanninkhof (1992) using the root mean square of the wind speed measured during the time on station (typically 4 h).

2.2. Determination of rates of N_2 fixation

For the determination of the rate of N_2 fixation, at each CTD or underway station, 25 L of water was collected from 15 m or 7 m, respectively. Four 4.5 L polycarbonate incubation bottles were rinsed three times and filled to overflowing before being sealed with a silicon septum screw cap. The cap was carefully placed on each bottle, ensuring that no air bubbles were introduced to the sample. $^{15}N_2$ gas (3 mL of 98 atom %, Isotec isotopes) was injected through the septum cap of each bottle with a gas-tight syringe. Each set of four 4.5 L bottles was placed in an on-deck incubator adjusted to light conditions in the depth region of sampling and cooled using surface seawater from the ship's seawater supply. After 24 hours, suspended particles were gently filtered from each bottle by positive pressure filtration. The contents of two bottles were each filtered directly onto a pre-combusted (450 °C for 4 hours) 25 mm GF/F filter, while the contents of the remaining two bottles were filtered through a 10 μm polycarbonate filter prior to filtration through a GF/F. Filters were immediately frozen at −20 °C in an onboard freezer. In the laboratory, filters were dried at 60 °C for 24 hours and then encapsulated in tin cups for analysis of their ^{15}N composition using a Carlo Erba NC2500 elemental analyzer interfaced to a Delta S isotope ratio mass spectrometer. Rates of net N_2 fixation ($\mu mol\,N\,m^{-3}\,d^{-1}$) for the whole community and the less than 10 μm size fraction were calculated according to methods described by Montoya et al. (1996).

3. Results and discussion

3.1. Validation of sampling for dissolved H_2

An important component of the study was determining the quality of the samples drawn from the ship's seawater supply as it was anticipated that H_2 contamination might result even from very low rates of corrosion in the plumbing system. Validation of the pumped supply was made difficult by the need for comparisons to be made with water collected at the same time but by different means. Ideal conditions are virtually impossible to achieve, since the ship's supply is best used with the vessel in motion, when water in the inlet zone is being continuously refreshed, but Niskin or bucket samples must be taken with the ship stationary. It was found that samples taken from the ship's seawater supply close to the intake contained much higher concentrations of H_2 than in samples drawn from the laboratory tap. This is illustrated in Fig. 2 from which it was determined that a factor of 3.5 needed to be applied to the samples drawn from the laboratory tap to correct for H_2 loss. The regression equation was: $[H_2]$ in uncontaminated supply $= 0.009+0.243 \times [H_2]$non-piped; $r^2 = 0.86$). A possible explanation of the loss is removal of H_2 by a biofilm, though the process must be rapid since the residence time of water in the plumbing is on the order of 2 minutes. The fact that the H_2 concentration was not depleted immediately downstream of the supply pump indicates that cavitation in the pump is unlikely to be responsible for H_2 loss. Furthermore, the fact that H_2 removal in the plumbing was large enough to bring concentrations to below saturation with the atmosphere argues against continuous transfer into bubbles or headspace within the

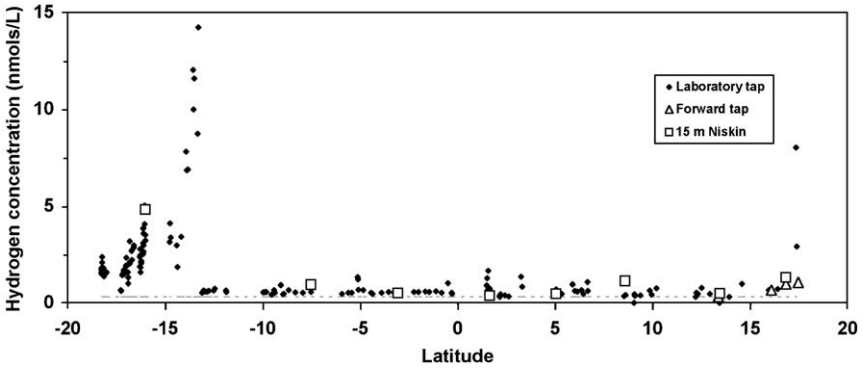


Fig. 3. Variation of dissolved H_2 along the cruise track (corrected for losses in the case of samples from the aft laboratory).

system and supports the idea of true consumption. It will be shown that with the correction factor applied, there is reasonable agreement between the shallowest (15 m) Niskin samples and those taken by pumping in the same vicinity (Fig. 3). It is important to note that applying a constant correction factor to the transit samples would not alter any correlation between measured H_2 and N_2 fixation rate. Nonetheless, it is not known whether the loss rate has a first order dependence on H_2 concentration, and clearly it is desirable to minimize or eliminate this source of interference.

3.2. Dissolved H_2 concentrations and saturation states

The variation of dissolved H_2 in surface waters with latitude along the cruise track is shown in Fig. 3. Dissolved H_2 concentrations ranged from 0.3 to 12.6 nmol L^{-1}. In addition to the corrected concentrations for the uncontaminated supply, results are also shown for samples taken close to the intake of the ship's uncontaminated supply and for 15 m Niskin bottles. The concentration corresponding to equilibrium with the atmosphere (0.33–0.34 nmol L^{-1}) is indicated by a broken line. It is apparent that large, increasing supersaturations were seen during the early part of the transect, with a precipitous decline at ca. 13°S to near saturation. The most northerly samples show evidence of a return to significant supersaturation. Evidence that the dramatic decrease was not a result of the sudden removal of a source of contamination comes from profile measurements given below. A similar steep change was reported by Setser et al. (1982) in the Pacific off Baja California. In that case, the H_2 hovered around saturation for several hours and then suddenly increased to over 20 nmol L^{-1} as the ship crossed a front (from cold to warm water with a simultaneous increase in fluorescence).

For comparison, the depth profiles are shown in Fig. 4. Since these samples are independent of the ship's supply, they are able to provide support for the surface distribution of H_2 that has already been described. Particularly notable are the high H_2 concentrations measured in samples from 15 m at Station 1. The decrease in concentration seen in every profile below the upper 100 m is interpreted as resulting from bacterial uptake and declining production with depth. Similar concentration decreases with depth are seen commonly, for example, in high latitude waters (Herr, 1984; Punshon et al., 2007), and mid to low latitude waters in the Atlantic (Schropp et al., 1987), and Mediterranean (Scranton et al., 1982). Higher concentrations of H_2 in the euphotic zone are consistent with the dependence of N_2 fixation on light energy. Bacterial consumption is well known in soils where it accounts for the largest sink for atmospheric H_2, and evidence has been provided for its occurrence in marine waters (Punshon et al., 2007). The absence of spikes in the deep samples argues that the Niskin samples are not significantly contaminated. At Station 1, a peak in H_2 is seen at 150 m, which is the depth corresponding to the deep chlorophyll-a maximum; nitrate plus nitrite remained extremely low at 0.01 μmol kg^{-1} at this depth (Fig. 5a). Although N_2 fixation rates were not determined in the deep chlorophyll maximum on this transect, previous studies in the subtropical Pacific have measured significant rates of N_2 fixation at depths of 120 m (Montoya et al., 2004), with pre-incubation size exclusion of colonial diazotrophs allowing the observed fixation to be attributed to unicellular cyanobacteria. Thus, the peak in H_2 at 150 m at station 1 may be due to N_2 fixation by unicellular cyanobacteria in the deep chlorophyll maximum. Nitrogenase *nifH* gene copy abundance shows that *Crocosphaera* was present at this station and had a copy abundance of ca. 10^3 L^{-1} in the depth range 100–150 m (M. Church, personal communication) – a value that is typical of near surface water concentrations near Station ALOHA in the oligotrophic North Pacific Ocean (Church et al., 2005).

3.3. Rates of N_2 fixation

The $^{15}N_2$ uptake technique is reported to measure net rates of N_2 fixation (Mulholland, 2007). Data from 8 CTD stations (15 m) and 6 underway stations (7 m) are shown in Fig. 6). For each site sampled (station and underway) the net rate of N_2 fixation is given for the whole biological community and for the diazotrophs smaller than 10 μm. Net N_2 fixation rates for the whole community ranged from 0.18 to 9.0 μmol N m^{-3} d^{-1} and were highest at the southern (~16°S) and northern (17°N) ends of the

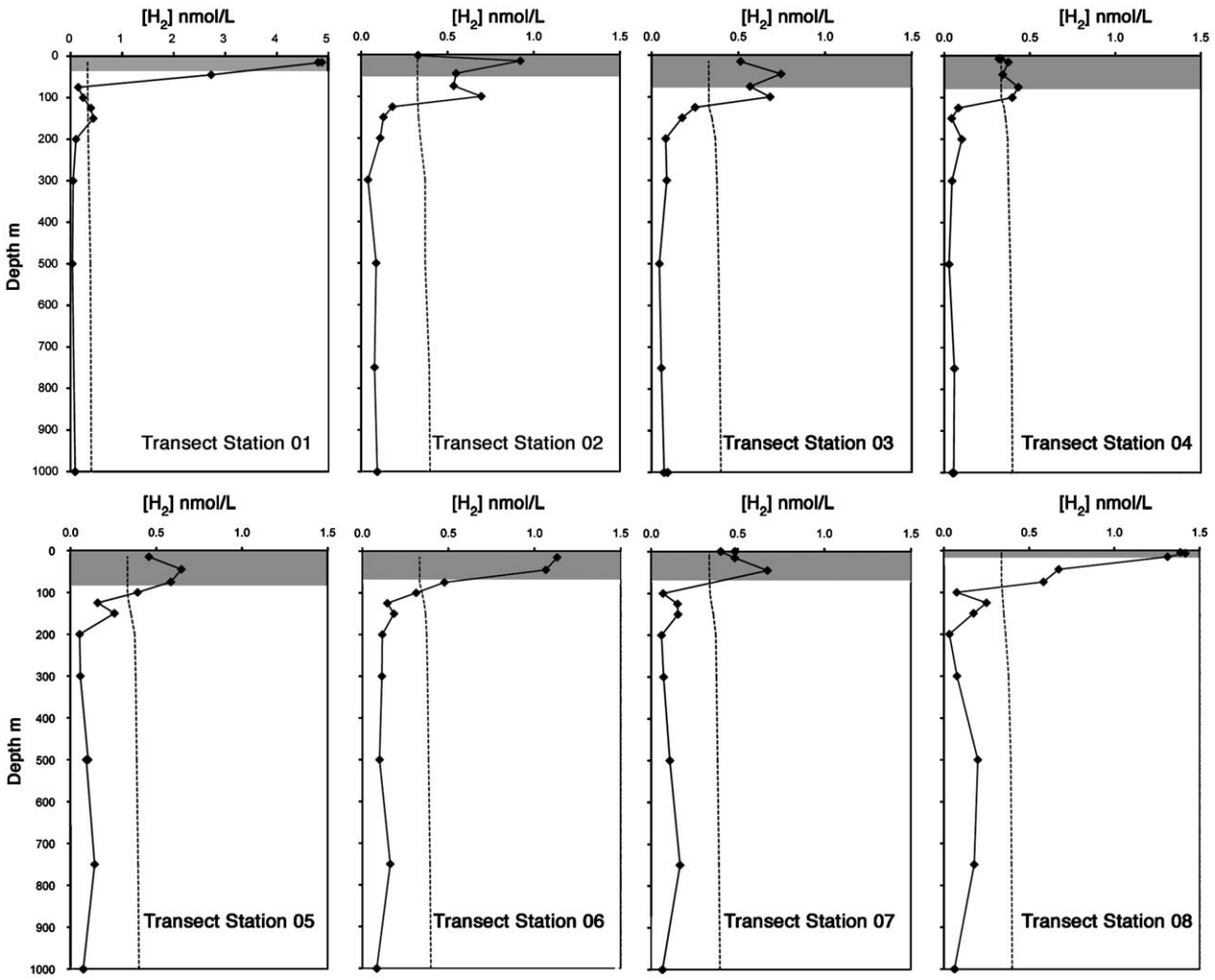


Fig. 4. Vertical profiles of dissolved H_2. The broken line denotes the solubility profile of H_2. The mixed layer depths are indicated by shading. Note the different concentration scale for Station 1.

transect. The very high rate of N_2 fixation and high supersaturation of H_2 at the southernmost station was consistent with genomic data showing a high abundance of the unicellular diazotrophic cyanobacterium, *Crocosphaera* (M. Church, personal communication). The $<10\,\mu m$ size fraction contributed 36% to 100% to the community N_2 fixation rates, which ranged from 0.17 to $5.1\,\mu mol\,N\,m^{-3}\,d^{-1}$. It is worth noting that the $^{15}N_2$ technique may lead to overestimation of the net rates of N_2 fixation by the whole community and the $<10\,\mu m$ size fraction of the community due to exudation of ^{15}N-labelled ammonium or DON by diazotrophs and recycling of these nitrogen sources by non-N_2 fixing microbes.

3.4. Comparison of H_2 concentrations and rates of N_2 fixation

It is clear that qualitatively there is a close similarity in the pattern of N_2 fixation and dissolved H_2 with the possible exception of the northernmost station (Figs. 3 and 6). For the station samples, it is straightforward to compare the H_2 concentration with the rate of N_2 fixation because the measurements were made on water from the same depths and location and there is no evidence for addition or removal of H_2 by the Niskin bottles used. In contrast, measurements from the underway stations are less ideal for comparison because the N_2 fixation rate was determined from water collected over the course of 30 minute's steaming, while the corresponding H_2 samples required only about 1 minute to collect. Furthermore, for these samples it is necessary to apply a correction to the H_2 concentration for loss in the plumbing. Because the N_2 fixation rates measured along this transect were mainly clustered around low values, the station data have been supplemented with the one underway sample from 15°S where the N_2 fixation rate was high, and one additional point from subsequent Niskin bottle sampling at Station ALOHA (Fig. 7).

It is apparent from this limited data set that the dissolved H_2 concentration shows a significant correlation

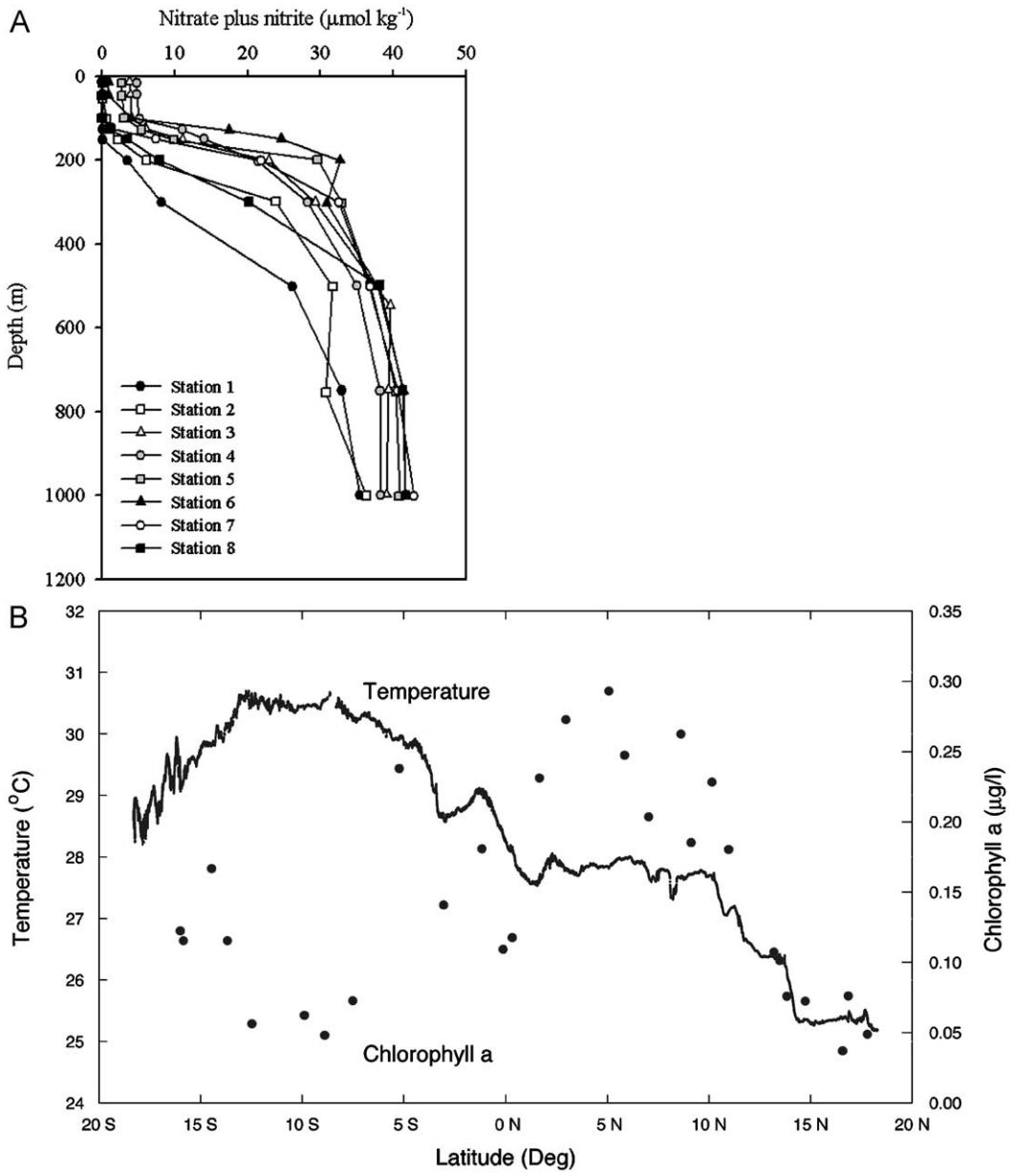


Fig. 5. A. Station profiles of nitrate plus nitrite (μmol kg^{-1}) from 0 to 1000 m. B. Surface temperatures and chlorophyll-a concentrations as a function of latitude.

with the rate of net N_2 fixation as measured by $^{15}N_2$ uptake (N_2 fixation $= -0.15+1.73$ $[H_2]_{excess}$, correlation coefficient, 0.96, Fig. 7). This result was determined by using a weighted regression that allowed for the additional uncertainty in dissolved H_2 concentration for the underway sample (weighting for that sample: 0.07 compared with 1 for all other points).

More persuasive would be a demonstration of correlation between the net biological production of H_2 and $^{15}N_2$ uptake rate. Under ideal conditions it would be possible to calculate the net H_2 production rate in the surface mixed layer by assuming that it is in steady state balance with loss to the atmosphere. We must also assume that there is no significant exchange of H_2 across the base of the mixed layer due to vertical mixing, and that the mixed layer depth is unchanging. Additionally, in the absence of continuous wind speed measurements during a period of days to weeks preceding sampling, either a constant spot wind speed, or a climatological value, must be used to calculate the exchange velocity for H_2. Using the wind speed measured at the time of each station (see Methods), we present estimates of the net rate of H_2 production (Table 1). The production rate is that required throughout the mixed layer to balance the efflux to the atmosphere, and is given by the product of the exchange velocity and the difference between the observed concentration of H_2

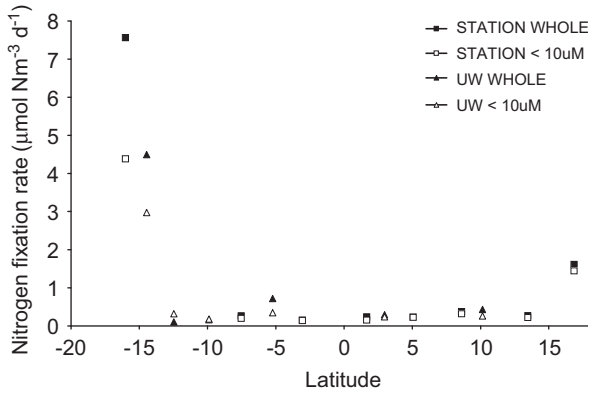


Fig. 6. Latitudinal variation in rates of N_2 fixation ($\mu mol\,N\,m^{-3}\,d^{-1}$). Samples were collected from the CTD (15 m, square) and from the uncontaminated seawater supply (triangles). Rates are reported for whole community (filled squares and triangles) and the $<10\,\mu m$ size fraction (unfilled square and triangles).

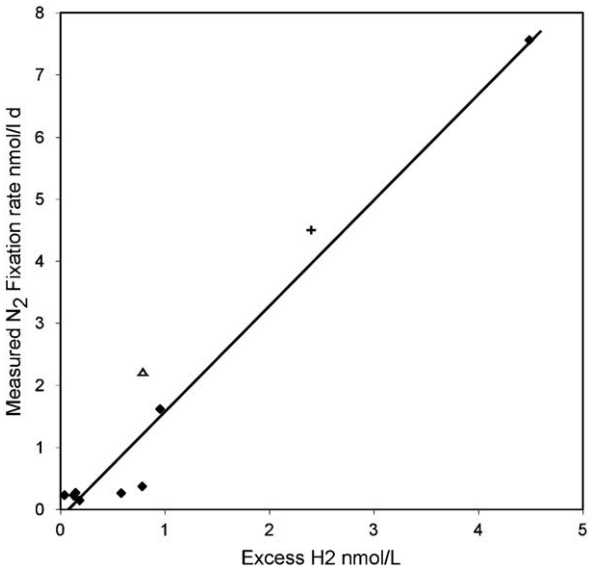


Fig. 7. Nitrogen fixation rate plotted against excess H_2 concentration: N_2 fixation $= -0.15+1.73$ [H_2] excess, correlation coefficient 0.96. Diamonds represent samples collected at stations along the Fiji-Hawaii transect; the cross represents an underway sample from 15°S, and the triangle, a sample from the Hawaii Ocean Time Series station.

in the mixed layer and the concentration that would exist at equilibrium with the atmosphere.

These rates have then been used to calculate a ratio of net H_2 production per mole of N fixed. If the $^{15}N_2$ method accounted fully for nitrogen fixed, and if there were no biological uptake of H_2, 0.5 mole of H_2 would be released for each mole of N fixed. Highly variable values of this ratio were found – low for station 1 where the highest rates of N_2 fixation and H_2 supersaturation were measured, and an order of magnitude higher at Station 6 where H_2 was only moderately supersaturated and N_2 fixation was low. The ratio will be indeterminate in a case where the N_2 fixation rate, or the degree of H_2 supersaturation was too low to measure. Such is the case for Station 4, where dissolved H_2 was close to equilibrium with the atmosphere. The variability means that the estimated rate of net H_2 production is not correlated with the measured N_2 fixation rate. For the purpose of illustrating some of the uncertainties in these calculations, a value for the ventilation time for H_2, estimated from the ratio of the mixed layer depth (m) to the exchange velocity ($m\,d^{-1}$), is given (column 5 of Table 1). The fact that the ventilation time may be up to 3 weeks, highlights the inadequacies of a calculation based on the assumption of steady state: neither wind speed nor mixed layer depth are likely to be constant for the duration of the longer ventilation times. A further significant source of uncertainty in these calculations arises from the criteria selected for determining the mixed layer depth: relaxing the criteria can substantially increase the mixed layer depth and reduce the calculated net H_2 production rate. It is concluded that the net rate of H_2 production cannot be reliably calculated without the unjustifiable assumption of steady state and the introduction of major uncertainty in selection of the appropriate wind speed and mixed layer depth. Therefore this study cannot demonstrate that the net rate of H_2 production is correlated with the rate of net N_2 fixation.

Table 1
Estimates of net H_2 production based on steady-state air-sea exchange and comparison with nitrogen fixation rates.

Station	RMS windspeed (m/s)	Exchange velocity (m/day)	Mixed layer depth (m)	Ventilation time (days)	Net H_2 production rate (μmol/m^3 d)	Mol H_2/mol N
1	4.9	3.8	36	10	0.47	0.06
2	4.6	3.4	53	15	0.04	0.14
3	6.3	6.0	78	13	0.01	0.15
4	5.2	4.0	80	20	0.00	–
5	9.6	13.7	83	6	0.02	0.09
6	11.3	19.0	67	4	0.22	0.59
7	11.1	17.9	67	4	0.04	0.29
8	8.6	10.5	17	2	0.59	0.37

The ventilation time of H_2 in the mixed layer (column 5) was estimated from mixed layer depth/exchange velocity. Column 7 gives the number of H_2 molecules released per atom of N fixed.

It is worth paying some attention to the processes underlying the net production of H_2 in ocean waters. It is not known how the rate of production of H_2 normalized to N fixed may vary amongst various groups of diazotrophs, or amongst different species or ecotypes of a single genus, such as *Trichodesmium* which can occur as free trichomes (single filaments of cells about 100–200 cells long), or in one of two characteristic colony morphologies (tufts or puffs). At present there remains uncertainty as to the identity of some of the marine diazotrophs. (For example, at Station 8 the highest *nifH* gene copy abundance was associated with an uncultivated unicell, M. Church, personal communication.) It is known that certain bacteria can utilize H_2 as an energy source, and it is possible that diazotrophs may themselves recycle H_2. In addition we would expect some degree of variability in the rate at which H_2 is utilized microbially. Amongst the

most obvious sources of such variability would be spatial variation in the abundances, species, and diversity of H_2-consumers. However, to the extent that the utility of a relationship between H_2 and N_2 fixation lies in the ease with which H_2 can be measured easily from a moving vessel, it is the empirical relationship between N_2 fixation and excess H_2 rather than H_2 production rate that is important. This is because the mixed layer depth, needed for the mass balance calculation, is a quantity that cannot readily be determined underway.

Since the existence of a correlation between H_2 supersaturation and the rate of N_2 fixation (Fig. 7) does not itself prove any causal relationship, some attention will be given to evidence for a link between the two processes and to the question of other possible sources of H_2 in ocean waters. In spite of the lack of a full and detailed understanding of the mechanism whereby it occurs, the liberation of H_2 by nitrogenase during N_2 fixation, and at greater rates when N_2 and other substrates such as CO and acetylene are absent, is well established (e.g. Hadfield and Bulen, 1969; Burgess and Lowe, 1996). Scranton (1983) demonstrated H_2 production in cultures of the diazotroph, *Trichodesmium*, though no correlation was found with the rate of acetylene reduction. Punshon and Moore (2008a) have observed production of H_2 in cultures of *Trichodesmium erythraeum* IMS101 that were actively fixing N_2, with a yield of 0.15–0.48 moles of H_2 evolved per mole of N_2 reduced. Herr et al. (1984) reported diurnal variation in dissolved H_2 in surface waters of the tropical South Atlantic with concentrations increasing during the morning and declining soon after local noon. They considered that biological production was the most likely explanation, but photochemical production was a possibility; N_2 fixation was not measured. Discriminating between photochemistry and N_2 fixation as potential sources of H_2 in the field is complicated by the fact that both processes are light dependent. While evidence has recently been provided for such a photochemical source of H_2, particularly in highly coloured waters (Punshon and Moore, 2008b), a case can be made for the influence of biological processes in the current study. Since it is not reasonable to attribute the major peak and abrupt decline in H_2 supersaturation seen at 14°S to variations in light intensity, any influence of photochemistry would have to be via a change in the concentration of an organic precursor of photo-produced H_2: this would in turn suggest the involvement of biology. The principle of parsimony favours the interpretation that the primary cause for the high concentrations of dissolved H_2 around 14°S is N_2 fixation.

4. Conclusions

Data collected in the equatorial Pacific between Fiji and Hawaii show that $^{15}N_2$-derived rates of N_2 fixation are correlated with the concentration of H_2 in excess of atmospheric solubility. However, an attempt to calculate the net rate of H_2 production by assuming a steady state between production and loss to the atmosphere yielded widely differing values for the ratio of net H_2 produced to net N_2 fixed. It is suggested that the reason for this is that, with a mixed layer ventilation time in the range of 2 to 20 days, steady state cannot be assumed; in particular, with a quadratic dependence of the exchange velocity on wind speed, the rate of H_2 loss cannot be assumed to be unchanging. Additional error will result from fluctuations of the mixed layer depth and the rate at which N_2 fixation is occurring.

Despite these discrepancies, continuous monitoring of the horizontal variation in H_2 concentrations in the tropical and subtropical regions of the ocean may shed light on the spatial extent of diazotroph activity, providing real-time data to guide sampling for N_2 fixation. In this study, we identified a sharp boundary between an area of intense net H_2 production and one in which production was minimal, implying patchiness in diazotroph activity. Such resolution cannot be achieved using more conventional techniques ($^{15}N_2$ or acetylene reduction). In addition, identifying the basin-scale distribution of N_2 fixation over various seasonal scales may help to constrain the area over which geochemical proxies for N_2 fixation (e.g. N*, Gruber and Sarmiento, 1997) are integrated (Mahaffey et al., 2005).

In this study it was found that major loss of H_2 occurred in the ship's seawater supply; it is suggested that this surprisingly large effect was the result of microbial processes. Future studies aiming at continuous sampling for H_2 need to find methods to correct or circumvent this problem.

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