

23 observed in the equatorial region between 8°S and 14°N. We propose that an empirical
24 relationship between H₂ supersaturations and N₂ fixation measurements could be used to
25 guide sampling for ¹⁵N fixation measurements or to aid the spatial interpolation of such
26 measurements.

27
28

29 **1. Introduction**

30
31 The existence of loss processes for combined nitrogen in the ocean, in particular
32 denitrification, makes the counterbalancing process of biological N₂ fixation one of the most
33 important in maintaining marine primary production. Following from this, N₂ fixation may
34 over long time scales play a role in the distribution of CO₂ between the atmosphere and deep
35 ocean (e.g. Falkowski, 1997). In spite of this central role and decades of study of the process,
36 the magnitude and spatial and temporal variability of N₂ fixation remain inadequately
37 determined. The identity of the organisms responsible and their mode of operation continue to
38 be topics of intensive research (Church et al., 2005; Foster and Zehr, 2006). One of the
39 difficulties of identifying and quantifying N₂ fixation is that few of its effects can be measured
40 quickly and directly. The uptake of elemental nitrogen itself is not easily measured against the
41 high background of dissolved N₂ which is caused by its relatively high solubility and high
42 atmospheric abundance. Also, there are other processes causing deviations from saturation,
43 such as bubble injection and changes in temperature and atmospheric pressure. Nitrogen
44 fixation activity is now commonly quantified through ¹⁵N₂ incubations (Montoya et al., 1996)
45 and subsequent analysis by isotope ratio mass spectrometry. Although sensitive and selective,
46 this method is not normally found to be practical on board ship, so incubated samples are
47 stored for analysis ashore. The traditional acetylene reduction technique (Stewart et al., 1967,

48 Zehr and Montoya, 2007), which employs gas chromatography with a flame ionization
49 detector (GC-FID), lacks the sensitivity to be easily used on cyanobacteria at normal ocean
50 abundance, although recent method development may overcome these difficulties (Staal et al.,
51 2007). However, it is possible that hydrogen (H_2) release during N_2 fixation could leave a
52 measurable signal that would have some utility in the study of this process.

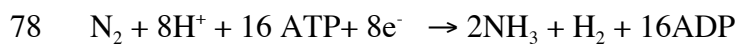
53 Hydrogen is a trace component of the atmosphere that is produced during the
54 photolysis of atmospheric formaldehyde, itself an intermediate in the oxidation of methane
55 initiated by reaction with OH (Simmonds et al., 2000). Other major sources are the burning of
56 biomass and fossil fuels (Novelli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

59 Hydrogen is typically supersaturated in low latitude surface waters with
60 concentrations that decrease exponentially below the mixed layer (Herr et al., 1984; Scranton
61 et al., 1982; Scranton, 1984; Conrad and Seiler, 1988). At higher latitudes supersaturations
62 decrease, and a few high latitude surveys show undersaturations in surface waters (Herr et al.,
63 1981, 1984; Punshon et al., 2007). It has long been recognized that there are two possible
64 sources of surface H_2 supersaturation. One is photochemical production which was suggested
65 to be responsible for an observed positive correlation between dissolved H_2 in surface waters
66 and solar irradiance in a study in the South Atlantic (Herr et al., 1984). A recent study of
67 photochemical production of H_2 in natural waters revealed that the effect was most
68 pronounced in highly coloured lake waters, but occurred also in coastal water samples
69 (Punshon and Moore, 2008b). No data were available for open ocean waters, but the study
70 suggested that some production would be expected based on a mechanism involving

71 photolysis of coloured dissolved organic matter (CDOM). The alternative, or complementary,
72 source from biological nitrogen fixation is better understood, although not all details of the
73 mechanism are known. It appears that the release of a H₂ molecule from dinitrogenase
74 (already in a partially reduced state) occurs with the uptake of a N₂ molecule prior to its
75 stepwise reduction to ammonia (Lowe and Thornely 1984; Postgate, 1998; Newton, 2007).

76 The stoichiometry of nitrogen reduction is given by:

77



79

80 Thus one H₂ is released for each N₂ reduced. However, H₂ can be oxidised through the action
81 of a hydrogenase and hence provide reducing power for the above reaction (Fisher and
82 Newton, 2002). While it may be expected that H₂, as an energy source, would be taken up,
83 either by the N₂-fixing organism itself, or by other members of the microbial community, a
84 signal of supersaturation should appear in the water, provided that the release and uptake are
85 not closely coupled. The level of supersaturation would depend on the rate of H₂ loss to the
86 atmosphere, and hence on wind speed, and the depth of the mixed layer in which production
87 is occurring as well as the strength of the microbial uptake sink. The magnitude of the
88 supersaturation is enhanced by the sparing aqueous solubility of H₂; for example, the
89 production of 1 nmol L⁻¹ of NH₃ (a typical daily fixation rate in tropical waters), if matched by
90 the stoichiometric release of 0.5 nmol L⁻¹ of H₂, would yield a saturation level of 240% in
91 water that was previously at equilibrium with the atmosphere at 25°C.

92 Production of H₂ during nitrogen fixation by the marine diazotroph, *Trichodesmium*,
93 has been demonstrated in laboratory experiments (Scranton 1983, Punshon and Moore,

94 2008a) and it has been recognized as a probable source of H₂ supersaturation in tropical
95 waters (Herr and Barger, 1978; Scranton 1983). With H₂ production being a fundamental part
96 of the mechanism of N₂ fixation by nitrogenases, it is to be expected that more recently
97 discovered marine diazotrophs would act as a source of H₂ in the ocean, but it cannot be
98 assumed that the ratio of its net release relative to N₂ fixed would be the same as for
99 *Trichodesmium*. We are not aware of any studies that have been made of H₂ release by the
100 unicellular cyanobacterium, *Crocospaera* (which has the ability to fix N₂ at night). Most
101 recently Zehr et al. (2009) have reported the existence of uncultivated unicellular marine
102 diazotrophic cyanobacteria that do not fix CO₂ or release O₂ since they lack photosystem II.
103 These organisms, free of inhibition of N₂-fixation by photosynthetic O₂, are also likely to play
104 a role in the marine H₂ cycle.

105

106 In the current study, dissolved H₂ was measured in an ocean transect, for the first time
107 paralleled by ¹⁵N₂ incubations to quantify N₂ fixation. The objectives of this study were to
108 determine whether N₂ fixation yields a significant transient signal of H₂ supersaturation in
109 surface ocean waters, and whether the signal may be correlated with the rate of N₂ fixation.
110 It is recognised that proceeding from any qualitative relationship to a quantitative one is likely
111 to be challenging because the concentration of H₂ in seawater is dependent on the balance
112 between a number of production and loss processes, rather than on a single production process
113 such as N₂ fixation. The loss processes in surface waters will include loss to the atmosphere at
114 a rate (per litre of water) that is dependent on wind speed, the degree of superaturation, and
115 the depth of the mixed layer (which determines the size of the exchangeable reservoir). In

116 addition to this physical loss, there can be a biological loss, which would be a function of the
117 species of H₂ consumers present, their abundance, and the state of their metabolism.

118

119 **2. Methods**

120 2.1 Hydrogen saturation

121 The work was done mainly during a transit of R/V Kilo Moana from Suva, Fiji to
122 Honolulu, Hawaii in April 2007 on the C-MORE sponsored BULA cruise (Figure 1);
123 reference is also made to work done on the same vessel during a cruise to the Hawaii Ocean
124 Times-Series (HOT) sampling site Station ALOHA (22° 45'N, 158°W), May 3-7, 2007. The
125 technique for measuring H₂, involving its GC separation from other reducing gases, oxidation
126 over heated HgO and detection by UV absorption is described in Punshon et al. (2007). In
127 brief, seawater samples were drawn through a long Teflon™ needle into a 50 mL glass
128 syringe fitted with a 3-way nylon stopcock and the sample volume adjusted to 35 mL. A 5 mL
129 headspace of zero air, free of H₂ and CO, was then introduced and the syringe shaken
130 vigorously for 3 minutes. This time period had previously been shown to result in complete
131 phase equilibration. The headspace was then transferred to the sample loop (1 mL) of a
132 reduction gas analyser (Peak Laboratories, USA). Phase equilibration was conducted at
133 ambient water temperature with the equilibration temperature for each sample being
134 determined from measurements made immediately before and after equilibration. Calibration
135 was effected using a 0.9 ppm primary standard prepared by gravimetric dilution of
136 volumetrically measured H₂ with ultra high purity N₂ in a 4.5 L internally electro-polished
137 stainless steel tank (Essex Cryogenics). Multi-point calibration curves were constructed daily
138 by diluting volumes of the primary standard with H₂-free zero air in a glass air sampling

139 syringe to produce H₂ mixing ratios in the range 0-0.9ppm. The coefficient of variation of the
140 analysis is $\pm 2\%$ and the detection limit 20 pmol L⁻¹. The solubility of H₂ was calculated using
141 the T and S dependent relationship of Weisenberg and Guinasso (1979).

142 During the transect there was frequent sampling from the ship's supply of seawater
143 which is delivered through a Teflon-lined stainless-steel pipe from a pump located in the
144 forward starboard hull drawing in water from a depth of 7 m. Samples were normally taken
145 from a seawater tap in an aft laboratory but several samples were also drawn from a tap
146 adjacent to this pump. Additionally, samples were taken from 10 L Niskin bottles during 8
147 vertical profiles. To minimize potential contamination from the rosette (resulting from
148 corrosion, e.g. of a zinc sacrificial anode), the bottles to be used for H₂ analysis were closed
149 during the upward profile as soon as the bottle reached the intended sampling depth.
150 (Potential sources of contamination and how they might be avoided have been discussed by
151 Scranton et al., 1982.) Water was fed into the bottom of an oxygen bottle (300 mL) via a
152 Tygon tube with the bottle being allowed to overflow by ~ 2 volumes before it was stoppered.
153 A few samples were taken with a plastic bucket and rope from the side of the ship with efforts
154 made to avoid water aerated by propeller wash. Duplicate samples were immediately drawn
155 from the bucket into 50 ml glass syringes. These samples, together with those taken near the
156 inlet to the ship's supply, are referred to as "non-piped" to distinguish them from the
157 laboratory tap samples that had passed through the ship's plumbing ("piped") (Figure 2).
158 Dissolved H₂ was measured typically within 1.5 hours.

159 The criteria for determining mixed layer depths were a rate of change in salinity and
160 temperature of 0.002 m⁻¹ and 0.01°C m⁻¹ respectively. Samples for determination of nitrate
161 and nitrite concentrations were collected from CTD stations at ten depths between 0 and

162 1000m. To determine surface ocean, low level nitrate plus nitrite concentrations (1 nmol kg^{-1}
163 to $1 \text{ } \mu\text{mol kg}^{-1}$), chemiluminescence techniques were employed using an Antek NO_x analyser
164 (Garside, 1982). For samples containing nitrate plus nitrite at concentrations $>1 \text{ } \mu\text{mol kg}^{-1}$,
165 standard continuous flow auto-analytical techniques were employed using a Bran and Luebbe
166 AA3 analyzer (Technicon, 1977). High-resolution temperature data were collected from the
167 ship's flow through seawater system using a SeaBird SBE-21 Seacat thermosalinograph.
168 Samples for measurement of chlorophyll-a concentrations ($\mu\text{g L}^{-1}$) were collected at CTD and
169 underway stations. Typically, 100 mL of water was collected and filtered immediately
170 through a 25 mm glass fiber filter (GF/F, $0.7\mu\text{m}$). The filter was stored and chlorophyll-a
171 extracted in 100% acetone at -20°C and concentrations determined using a Turner 10Au
172 fluorimeter (Strickland and Parsons, 1972). Exchange velocities used in the calculation of the
173 flux of H_2 between the ocean and atmosphere were determined from the relationship of
174 Wanninkof (1992) using the root mean square of the wind speed measured during the time on
175 station (typically 4 h).

176

177 2.2. Determination of rates of N_2 fixation

178 For the determination of the rate of N_2 fixation, at each CTD or underway station, 25 L
179 of water was collected from 15 m or 7 m, respectively. Four 4.5 L polycarbonate incubation
180 bottles were rinsed three times and filled to overflowing before being sealed with a silicon
181 septum screw cap. The cap was carefully placed on each bottle, ensuring that no air bubbles
182 were introduced to the sample. $^{15}\text{N}_2$ gas (3 mL of 98 atom %, Isotec isotopes) was injected
183 through the septum cap of each bottle with a gas-tight syringe. Each set of four 4.5 L bottles
184 was placed in an on-deck incubator adjusted to light conditions in the depth region of

185 sampling and cooled using surface seawater from the ship's seawater supply. After 24 hours,
186 suspended particles were gently filtered from each bottle by positive pressure filtration. The
187 contents of two bottles were each filtered directly onto a pre-combusted (450°C for 4 hours)
188 25 mm GF/F filter, while the contents of the remaining two bottles were filtered through a 10
189 µm polycarbonate filter prior to filtration through a GF/F. Filters were immediately frozen at -
190 20°C in an onboard freezer. In the laboratory, filters were dried at 60°C for 24 hours and then
191 encapsulated in tin cups for analysis of their ¹⁵N composition using a Carlo Erba NC2500
192 elemental analyzer interfaced to a Delta S isotope ratio mass spectrometer. Rates of net N₂
193 fixation (µmol N m⁻³ d⁻¹) for the whole community and the less than 10µm size fraction were
194 calculated according to methods described by Montoya *et al.* (1996).

195

196 **3. Results and discussion**

197 3.1. Validation of sampling for dissolved H₂

198 An important component of the study was determining the quality of the samples
199 drawn from the ship's seawater supply as it was anticipated that H₂ contamination might
200 result even from very low rates of corrosion in the plumbing system. Validation of the
201 pumped supply was made difficult by the need for comparisons to be made with water
202 collected at the same time but by different means. Ideal conditions are virtually impossible to
203 achieve, since the ship's supply is best used with the vessel in motion, when water in the inlet
204 zone is being continuously refreshed, but Niskin or bucket samples must be taken with the
205 ship stationary. It was found that samples taken from the ship's seawater supply close to the
206 intake contained much higher concentrations of H₂ than in samples drawn from the laboratory
207 tap. This is illustrated in Figure 2 from which it was determined that a factor of 3.5 needed to

208 be applied to the samples drawn from the laboratory tap to correct for H₂ loss. The regression
209 equation was: [H₂] in uncontaminated supply = 0.009 + 0.243 x [H₂]non-piped; r²=0.86). A
210 possible explanation of the loss is removal of H₂ by a biofilm, though the process must be
211 rapid since the residence time of water in the plumbing is on the order of 2 minutes. The fact
212 that the H₂ concentration was not depleted immediately downstream of the supply pump
213 indicates that cavitation in the pump is unlikely to be responsible for H₂ loss. Furthermore, the
214 fact that H₂ removal in the plumbing was large enough to bring concentrations to below
215 saturation with the atmosphere argues against continuous transfer into bubbles or headspace
216 within the system and supports the idea of true consumption. It will be shown that with the
217 correction factor applied, there is reasonable agreement between the shallowest (15 m) Niskin
218 samples and those taken by pumping in the same vicinity (Figure 3). It is important to note
219 that applying a constant correction factor to the transit samples would not alter any correlation
220 between measured H₂ and N₂ fixation rate. Nonetheless, it is not known whether the loss rate
221 has a first order dependence on H₂ concentration, and clearly it is desirable to minimize or
222 eliminate this source of interference.

223

224 3.2. Dissolved H₂ concentrations and saturation states

225 The variation of dissolved H₂ in surface waters with latitude along the cruise track is
226 shown in Figure 3. Dissolved H₂ concentrations ranged from 0.3 to 12.6 nmol L⁻¹. In addition
227 to the corrected concentrations for the uncontaminated supply, results are also shown for
228 samples taken close to the intake of the ship's uncontaminated supply and for 15 m Niskin
229 bottles. The concentration corresponding to equilibrium with the atmosphere (0.33-0.34 nmol
230 L⁻¹) is indicated by a broken line. It is apparent that large, increasing supersaturations were

231 seen during the early part of the transect, with a precipitous decline at ca. 13°S to near
232 saturation. The most northerly samples show evidence of a return to significant
233 supersaturation. Evidence that the dramatic decrease was not a result of the sudden removal of
234 a source of contamination comes from profile measurements given below. A similar steep
235 change was reported by Setser et al. (1982) in the Pacific off Baja California. In that case, the
236 H₂ hovered around saturation for several hours and then suddenly increased to over 20 nmol
237 L⁻¹ as the ship crossed a front (from cold to warm water with a simultaneous increase in
238 fluorescence).

239 For comparison, the depth profiles are shown in Figure 4. Since these samples are
240 independent of the ship's supply, they are able to provide support for the surface distribution
241 of H₂ that has already been described. Particularly notable are the high H₂ concentrations
242 measured in samples from 15 m at Station 1. The decrease in concentration seen in every
243 profile below the upper 100 m is interpreted as resulting from bacterial uptake and declining
244 production with depth. Similar concentration decreases with depth are seen commonly, for
245 example, in high latitude waters (Herr, 1984; Punshon et al., 2007), and mid to low latitude
246 waters in the Atlantic (Shropp et al., 1987), and Mediterranean (Scranton et al., 1982). Higher
247 concentrations of H₂ in the euphotic zone are consistent with the dependence of N₂ fixation on
248 light energy. Bacterial consumption is well known in soils where it accounts for the largest
249 sink for atmospheric H₂, and evidence has been provided for its occurrence in marine waters
250 (Punshon et al., 2007). The absence of spikes in the deep samples argues that the Niskin
251 samples are not significantly contaminated. At Station 1, a peak in H₂ is seen at 150m, which
252 is the depth corresponding to the deep chlorophyll-a maximum; nitrate plus nitrite remained
253 extremely low at 0.01 μmol kg⁻¹ at this depth (Figure 5a). Although N₂ fixation rates were not

254 determined in the deep chlorophyll maximum on this transect, previous studies in the
255 subtropical Pacific have measured significant rates of N₂ fixation at depths of 120m (Montoya
256 et al., 2004), with pre-incubation size exclusion of colonial diazotrophs allowing the observed
257 fixation to be attributed to unicellular cyanobacteria. Thus, the peak in H₂ at 150m at station 1
258 may be due to N₂ fixation by unicellular cyanobacteria in the deep chlorophyll maximum.
259 Nitrogenase *nifH* gene copy abundance shows that *Crocospaera* was present at this station
260 and had a copy abundance of ca. 10³ L⁻¹ in the depth range 100-150m (M. Church, personal
261 communication) – a value that is typical of near surface water concentrations near Station
262 ALOHA in the oligotrophic North Pacific Ocean (Church et al. 2005).

263

264 3.3. Rates of N₂ fixation

265 The ¹⁵N₂ uptake technique is reported to measure net rates of N₂ fixation (Mulholland,
266 2007). Data from 8 CTD stations (15m) and 6 underway stations (7m) are shown in Figure 6).
267 For each site sampled (station and underway) the net rate of N₂ fixation is given for the whole
268 biological community and for the diazotrophs smaller than 10µm. Net N₂ fixation rates for the
269 whole community ranged from 0.18 to 9.0 µmol N m⁻³ d⁻¹ and were highest at the southern (~
270 16°S) and northern (17°N) ends of the transect. The very high rate of N₂ fixation and high
271 supersaturation of H₂ at the southernmost station was consistent with genomic data showing a
272 high abundance of the unicellular diazotrophic cyanobacterium, *Crocospaera* (M. Church,
273 personal communication). The <10 µm size fraction contributed 36% to 100% to the
274 community N₂ fixation rates, which ranged from 0.17 to 5.1 µmol N m⁻³ d⁻¹. It is worth noting
275 that the ¹⁵N₂ technique may lead to overestimation of the net rates of N₂ fixation by the whole
276 community and the <10µm size fraction of the community due to exudation of ¹⁵N-labelled

277 ammonium or DON by diazotrophs and recycling of these nitrogen sources by non-N₂ fixing
278 microbes.

279

280 3.4. Comparison of H₂ concentrations and rates of N₂ fixation

281 It is clear that qualitatively there is a close similarity in the pattern of N₂ fixation and
282 dissolved H₂ with the possible exception of the northernmost station (Figures 3 and 6). For the
283 station samples, it is straightforward to compare the H₂ concentration with the rate of N₂
284 fixation because the measurements were made on water from the same depths and location
285 and there is no evidence for addition or removal of H₂ by the Niskin bottles used. In contrast,
286 measurements from the underway stations are less ideal for comparison because the N₂
287 fixation rate was determined from water collected over the course of 30 minute's steaming,
288 while the corresponding H₂ samples required only about 1 minute to collect. Furthermore, for
289 these samples it is necessary to apply a correction to the H₂ concentration for loss in the
290 plumbing. Because the N₂ fixation rates measured along this transect were mainly clustered
291 around low values, the station data have been supplemented with the one underway sample
292 from 15°S where the N₂ fixation rate was high, and one additional point from subsequent
293 Niskin bottle sampling at Station ALOHA (Figure 7).

294 It is apparent from this limited data set that the dissolved H₂ concentration
295 shows a significant correlation with the rate of net N₂ fixation as measured by ¹⁵N₂ uptake (N₂
296 fixation = -0.15 + 1.73 [H₂]_{excess}, correlation coefficient, 0.96, Figure 7). This result was
297 determined by using a weighted regression that allowed for the additional uncertainty in
298 dissolved H₂ concentration for the underway sample (weighting for that sample: 0.07
299 compared with 1 for all other points).

300 More persuasive would be a demonstration of correlation between the net biological
 301 production of H₂ and ¹⁵N₂ uptake rate. Under ideal conditions it would be possible to calculate
 302 the net H₂ production rate in the surface mixed layer by assuming that it is in steady state
 303 balance with loss to the atmosphere. We must also assume that there is no significant
 304 exchange of H₂ across the base of the mixed layer due to vertical mixing, and that the mixed
 305 layer depth is unchanging. Additionally, in the absence of continuous wind speed
 306 measurements during a period of days to weeks preceding sampling, either a constant spot
 307 wind speed, or a climatological value, must be used to calculate the exchange velocity for H₂.
 308 Using the wind speed measured at the time of each station (see Methods), we present
 309 estimates of the net rate of H₂ production (Table 1). The production rate is that required
 310 throughout the mixed layer to balance the efflux to the atmosphere, and is given by the
 311 product of the exchange velocity and the difference between the observed concentration of H₂
 312 in the mixed layer and the concentration that would exist at equilibrium with the atmosphere.

313

314 Table 1. Estimates of net H₂ production based on steady-state air-sea exchange and
 315 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

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The ventilation time of H₂ in the mixed layer (column 5) was estimated from mixed layer depth/exchange velocity. Column 7 gives the number of H₂ molecules released per atom of N fixed.

325 These rates have then been used to calculate a ratio of net H₂ production per mole of N
326 fixed. If the ¹⁵N₂ method accounted fully for nitrogen fixed, and if there were no biological
327 uptake of H₂, 0.5 mole of H₂ would be released for each mole of N fixed. Highly variable
328 values of this ratio were found – low for station 1 where the highest rates of N₂ fixation and
329 H₂ supersaturation were measured, and an order of magnitude higher at Station 6 where H₂
330 was only moderately supersaturated and N₂ fixation was low. The ratio will be indeterminate
331 in a case where the N₂ fixation rate, or the degree of H₂ supersaturation was too low to
332 measure. Such is the case for Station 4, where dissolved H₂ was close to equilibrium with the
333 atmosphere. The variability means that the estimated rate of net H₂ production is not
334 correlated with the measured N₂ fixation rate. For the purpose of illustrating some of the
335 uncertainties in these calculations, a value for the ventilation time for H₂, estimated from the
336 ratio of the mixed layer depth (m) to the exchange velocity (m d⁻¹), is given (column 5 of
337 Table 1). The fact that the ventilation time may be up to 3 weeks, highlights the inadequacies
338 of a calculation based on the assumption of steady state: neither wind speed nor mixed layer
339 depth are likely to be constant for the duration of the longer ventilation times. A further
340 significant source of uncertainty in these calculations arises from the criteria selected for
341 determining the mixed layer depth: relaxing the criteria can substantially increase the mixed
342 layer depth and reduce the calculated net H₂ production rate. It is concluded that the net rate
343 of H₂ production cannot be reliably calculated without the unjustifiable assumption of steady
344 state and the introduction of major uncertainty in selection of the appropriate wind speed and

345 mixed layer depth. Therefore this study cannot demonstrate that the net rate of H₂ production
346 is correlated with the rate of net N₂ fixation.

347

348 It is worth paying some attention to the processes underlying the net production of H₂
349 in ocean waters. It is not known how the rate of production of H₂ normalized to N fixed may
350 vary amongst various groups of diazotrophs, or amongst different species or ecotypes of a
351 single genus, such as *Trichodesmium* which can occur as free trichomes (single filaments of
352 cells about 100-200 cells long), or in one of two characteristic colony morphologies (tufts or
353 puffs). At present there remains uncertainty as to the identity of some of the marine
354 diazotrophs. (For example, at Station 8 the highest *nifH* gene copy abundance was associated
355 with an uncultivated unicell, M. Church, personal communication.) It is known that certain
356 bacteria can utilize H₂ as an energy source, and it is possible that diazotrophs may themselves
357 recycle H₂. In addition we would expect some degree of variability in the rate at which H₂ is
358 utilized microbially. Amongst the most obvious sources of such variability would be spatial
359 variation in the abundances, species, and diversity of H₂-consumers. However, to the extent
360 that the utility of a relationship between H₂ and N₂ fixation lies in the ease with which H₂ can
361 be measured easily from a moving vessel, it is the empirical relationship between N₂ fixation
362 and excess H₂ rather than H₂ production rate that is important. This is because the mixed layer
363 depth, needed for the mass balance calculation, is a quantity that cannot readily be determined
364 underway.

365

366 Since the existence of a correlation between H₂ supersaturation and the rate of N₂
367 fixation (Figure 7) does not itself prove any causal relationship, some attention will be given

368 to evidence for a link between the two processes and to the question of other possible sources
369 of H₂ in ocean waters. In spite of the lack of a full and detailed understanding of the
370 mechanism whereby it occurs, the liberation of H₂ by nitrogenase during N₂ fixation, and at
371 greater rates when N₂ and other substrates such as CO and acetylene are absent, is well
372 established (e.g. Hadfield and Bulen 1969; Burgess and Lowe, 1996). Scranton (1983)
373 demonstrated H₂ production in cultures of the diazotroph, *Trichodesmium*, though no
374 correlation was found with the rate of acetylene reduction. Punshon and Moore (2008a) have
375 observed production of H₂ in cultures of *Trichodesmium erythraeum* IMS101 that were
376 actively fixing N₂, with a yield of 0.15-0.48 moles of H₂ evolved per mole of N₂ reduced. Herr
377 *et al.* (1984) reported diurnal variation in dissolved H₂ in surface waters of the tropical South
378 Atlantic with concentrations increasing during the morning and declining soon after local
379 noon. They considered that biological production was the most likely explanation, but
380 photochemical production was a possibility; N₂ fixation was not measured. Discriminating
381 between photochemistry and N₂ fixation as potential sources of H₂ in the field is complicated
382 by the fact that both processes are light dependent. While evidence has recently been provided
383 for such a photochemical source of H₂, particularly in highly coloured waters (Punshon and
384 Moore, 2008b), a case can be made for the influence of biological processes in the current
385 study. Since it is not reasonable to attribute the major peak and abrupt decline in H₂
386 supersaturation seen at 14°S to variations in light intensity, any influence of photochemistry
387 would have to be via a change in the concentration of an organic precursor of photo-produced
388 H₂: this would in turn suggest the involvement of biology. The principle of parsimony favours
389 the interpretation that the primary cause for the high concentrations of dissolved H₂ around
390 14°S is N₂ fixation.

391 **4. Conclusions**

392 Data collected in the equatorial Pacific between Fiji and Hawaii show that $^{15}\text{N}_2$ -
393 derived rates of N_2 fixation are correlated with the concentration of H_2 in excess of
394 atmospheric solubility. However, an attempt to calculate the net rate of H_2 production by
395 assuming a steady state between production and loss to the atmosphere yielded widely
396 differing values for the ratio of net H_2 produced to net N_2 fixed. It is suggested that the reason
397 for this is that, with a mixed layer ventilation time in the range of 2 to 20 days, steady state
398 cannot be assumed; in particular, with a quadratic dependence of the exchange velocity on
399 wind speed, the rate of H_2 loss cannot be assumed to be unchanging. Additional error will
400 result from fluctuations of the mixed layer depth and the rate at which N_2 fixation is
401 occurring.

402 Despite these discrepancies, continuous monitoring of the horizontal variation in H_2
403 concentrations in the tropical and subtropical regions of the ocean may shed light on the
404 spatial extent of diazotroph activity, providing real-time data to guide sampling for N_2
405 fixation. In this study, we identified a sharp boundary between an area of intense net H_2
406 production and one in which production was minimal, implying patchiness in diazotroph
407 activity. Such resolution cannot be achieved using more conventional techniques ($^{15}\text{N}_2$ or
408 acetylene reduction). In addition, identifying the basin-scale distribution of N_2 fixation over
409 various seasonal scales may help to constrain the area over which geochemical proxies for N_2
410 fixation (e.g. N^* , Gruber and Sarmiento, 1997) are integrated (Mahaffey et al., 2005).

411 In this study it was found that major loss of H_2 occurred in the ship's seawater supply;
412 it is suggested that this surprisingly large effect was the result of microbial processes. Future

413 studies aiming at continuous sampling for H₂ need to find methods to correct or circumvent
414 this problem.

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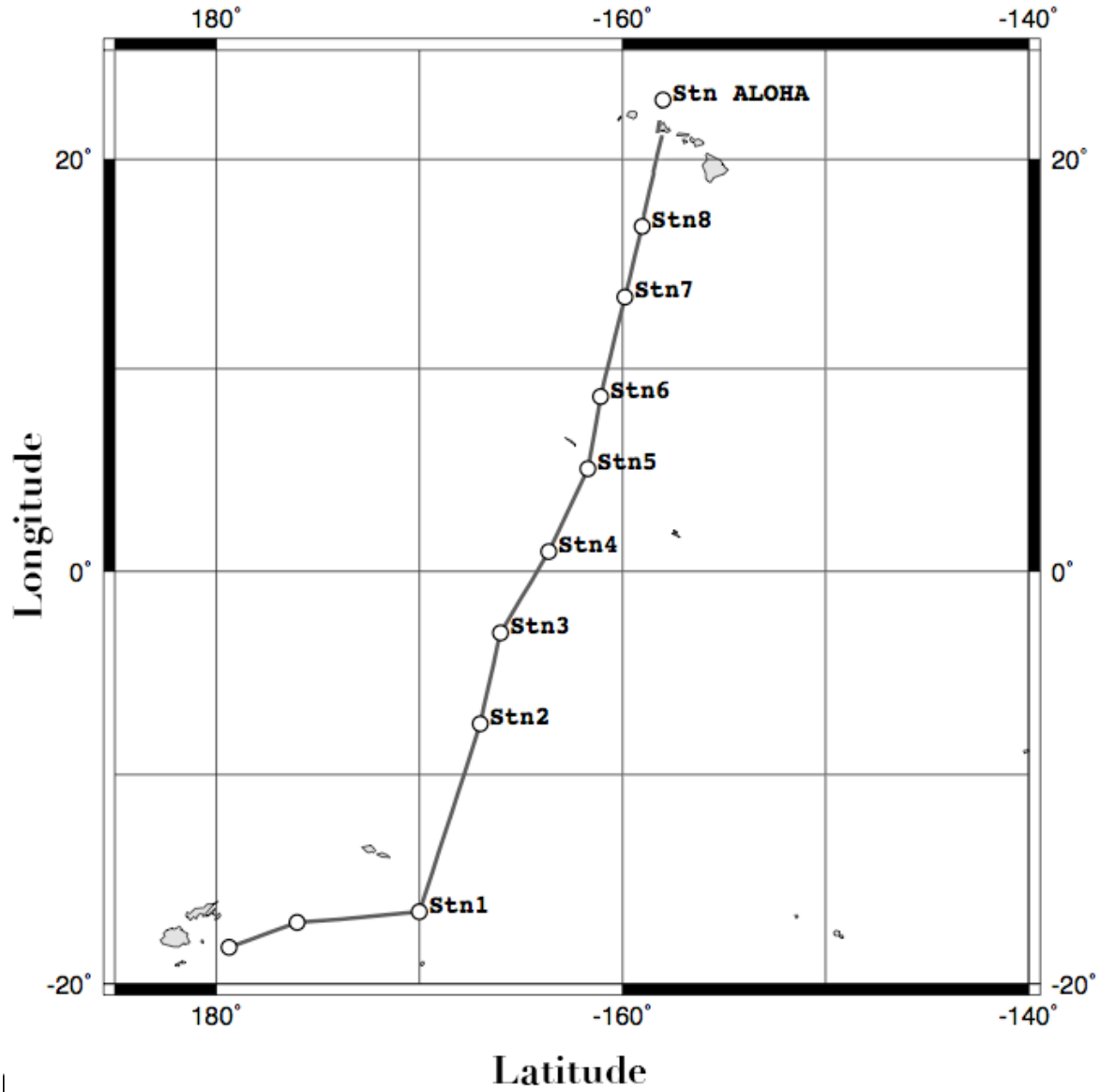
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513

514 **Figures**



515

516

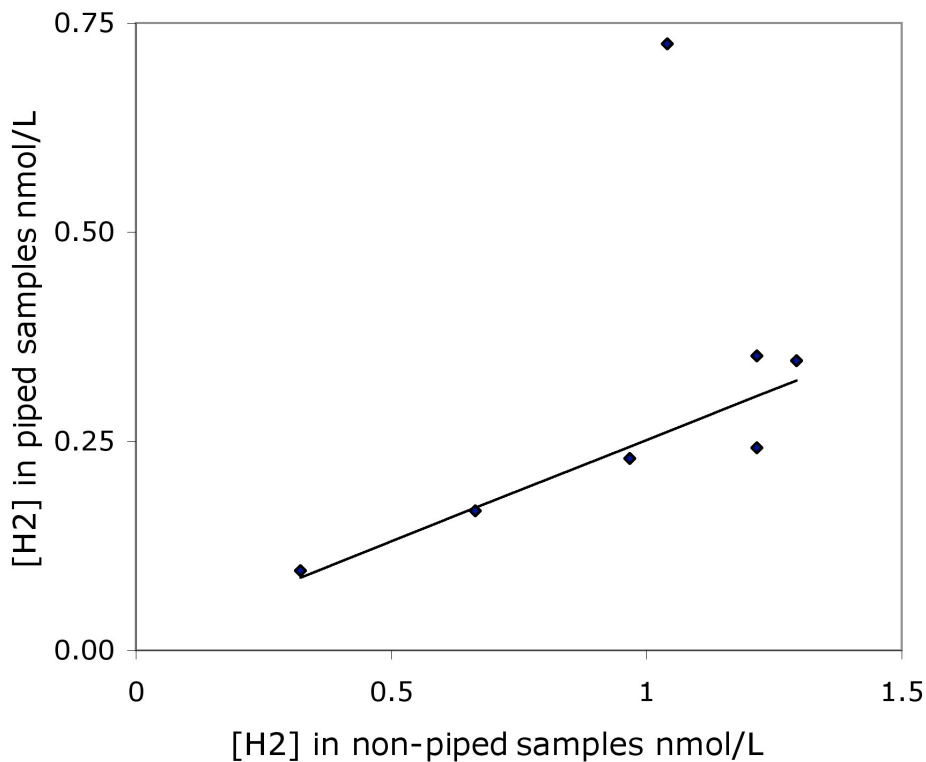
517 Figure 1. Cruise track of Kilo Moana showing station locations and the position of the Hawaii

518 Ocean Time Series station.

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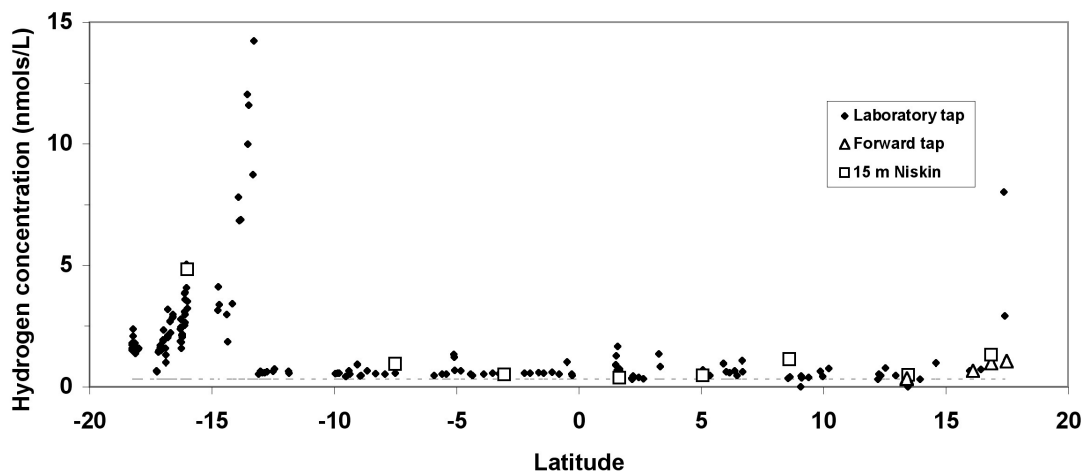
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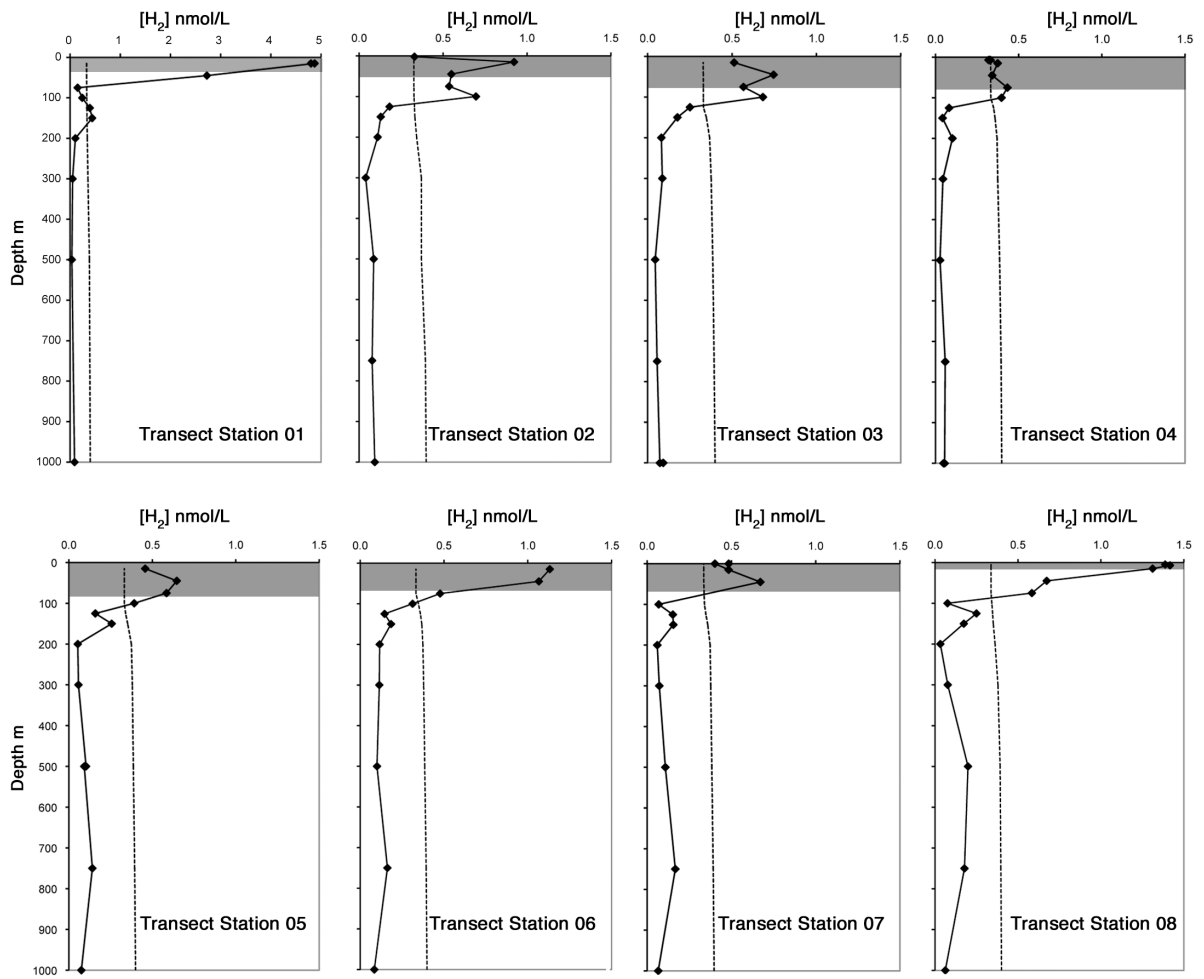
522

523 Figure 2. Plot of H₂ in water from ship's uncontaminated supply ("piped" samples) plotted
524 against values for samples that did not pass through the ship's plumbing. The regression line
525 ($y = 0.009 + 0.243x$; $r^2 = 0.86$) excludes the single outlying data point.



526

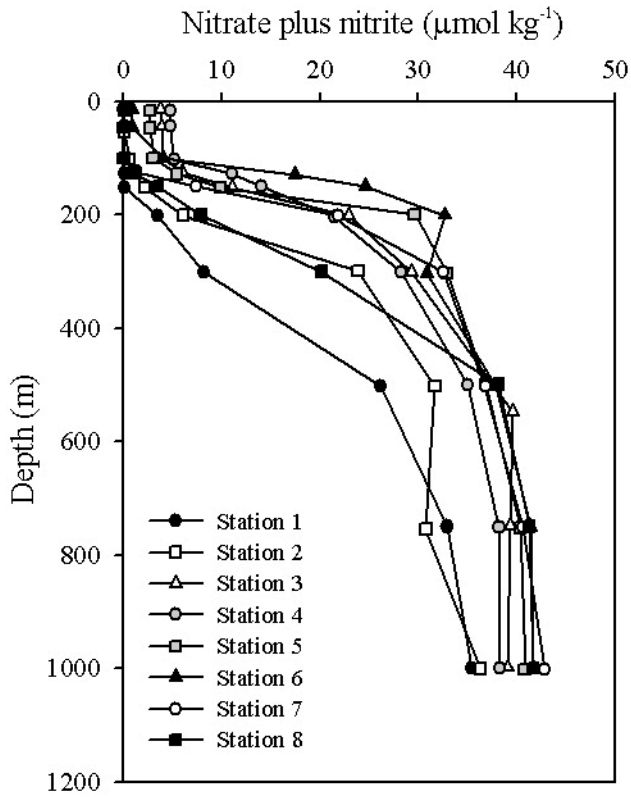
527 Figure 3. Variation of dissolved H_2 along the cruise track (corrected for losses in the case of
528 samples from the aft laboratory).



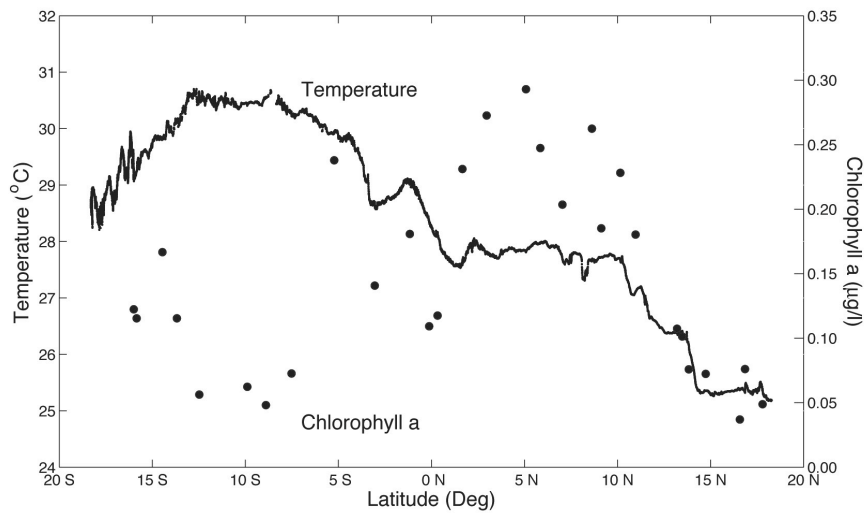
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530 Figure 4. Vertical profiles of dissolved H_2 . The broken line denotes the solubility profile of
531 H_2 . The mixed layer depths are indicated by shading. Note the different concentration scale
532 for Station 1.

533



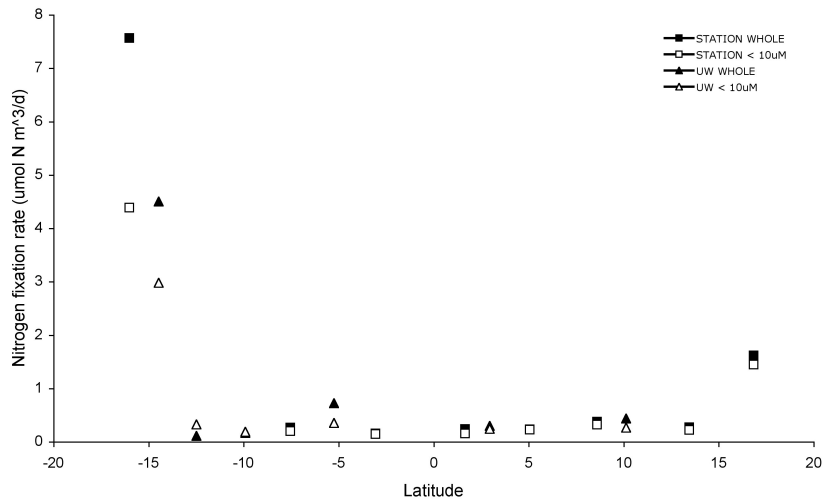
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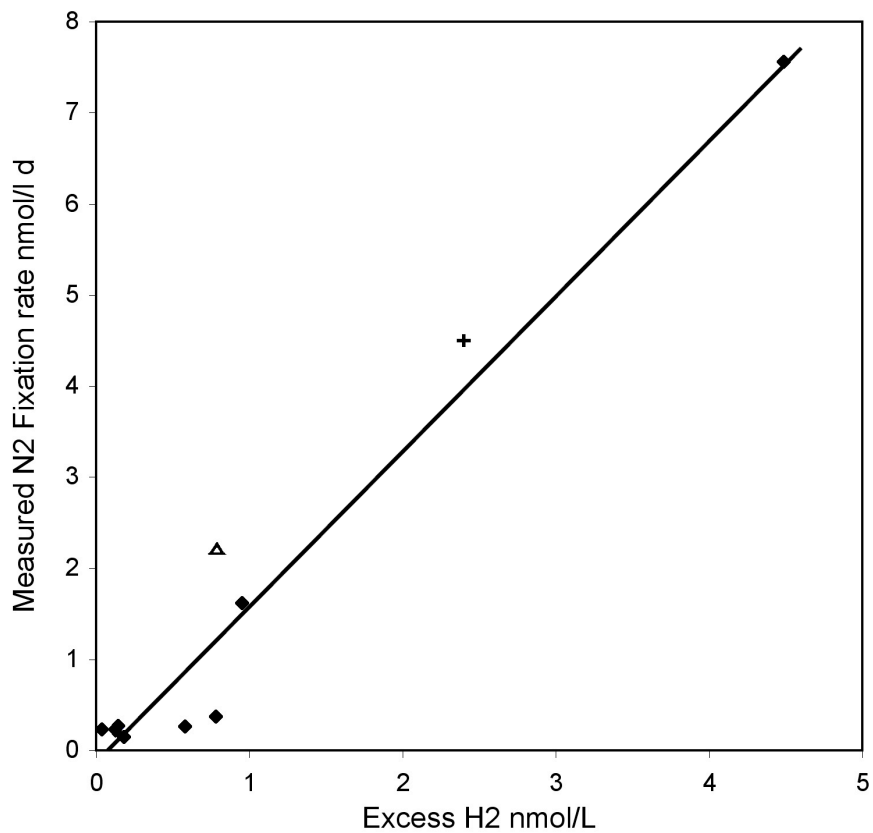
536 Figure 5. A. Station profiles of nitrate plus nitrite ($\mu\text{mol kg}^{-1}$) from 0 to 1000m. B Surface

537 temperatures and chlorophyll-a concentrations as a function of latitude.



539

540 Figure 6. Latitudinal variation in rates of N₂ fixation (µmol N m⁻³ d⁻¹). Samples were collected
 541 from the CTD (15m, square) and from the uncontaminated seawater supply (triangles). Rates
 542 are reported for whole community (filled squares and triangles) and the <10µm size fraction
 543 (unfilled square and triangles).



544

545 Figure 7. Nitrogen fixation rate plotted against excess H₂ concentration: N₂ fixation = -0.15 +
 546 1.73 [H₂] excess, correlation coefficient 0.96. Diamonds represent samples collected at
 547 stations along the Fiji-Hawaii transect; the cross represents an underway sample from 15°S,
 548 and the triangle, a sample from the Hawaii Ocean Time Series station.