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The role of the cyanobacterium *Oscillatoria (Trichodesmium) thiebautii* in the marine hydrogen cycle*

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ABSTRACT: The persistence of hydrogen supersaturations throughout much of the world's oceans is evidence for the existence of a hydrogen source in the oceanic mixed layer. Recent experiments in the waters near St. Croix have demonstrated that the marine cyanobacterium *Oscillatoria thiebautii* produces hydrogen in significant quantities when incubated for short periods in shore-based incubators. Although the relationship between hydrogen production rates as determined from shore-based incubations and water-column hydrogen concentrations is complex, it appears that *O. thiebautii* at natural population densities can produce hydrogen in sufficient quantities to maintain hydrogen supersaturations typical of those commonly seen in the open ocean. Preliminary results also suggest that hydrogen production and nitrogen fixation may not be strongly coupled in this system.

INTRODUCTION

The importance of specific microbial processes in controlling oceanic reduced gas distributions has been a subject of much investigation in the past decade. Many reduced gases, including hydrogen, have been found to be present in the oceanic mixed layer in concentrations considerably in excess of that predicted from solubility considerations. This has resulted in speculations that *in situ* production of hydrogen (and other reduced gases) is important (Seiler and Schmidt, 1974; Herr and Barger, 1978; Scranton et al., in press). Observations of undersaturation of hydrogen at depth and in polar regions has indicated that consumption may also be a significant process in the oceans (Herr and Barger, 1978; Herr et al., 1981; Scranton et al., 1982.

Two possible sources for the excess hydrogen observed in the mixed layer have been suggested: hydrogen production during anaerobic degradation of organic matter, and hydrogen production via nitrogenase-catalyzed reduction of protons in conjunction with biological nitrogen fixation. It seems probable that anaerobic production of hydrogen in the mixed layer is not a process of major importance because it is unlikely that anaerobic microenvironments are abundant within the highly oxidizing oceanic mixed layer (e.g. Scranton, 1977). In addition, hydrogen concentrations in anaerobic environments are usually quite low (Bullister et al., 1982; Scranton and Loud, 1982; Scranton, in prep.). In contrast, hydrogen production during N_2-fixation appears to be more probable as a source of oceanic H_2. Hydrogen production has been observed in association with nitrogen fixation in all classes of N_2-fixing bacteria (Evans et al., 1981), and several pelagic nitrogen fixing organisms have been identified. These latter include several *Oscillatoria* (formerly *Trichodesmium*) species (e.g. Dugdale et al., 1961; Mague et al., 1974; Carpenter and McCarthy, 1975; Carpenter and Price, 1977; Mague et al., 1977), *Dichothrix fucicola*, an epiphyte on pelagic sargassum (Carpenter, 1972), *Richelia intracellularis* (e.g. Mague et al., 1974; Mague et al., 1977) and organisms which live in association with *Rhizoselenia* mats (Martinez et al., 1982).

The net release of hydrogen from a cell appears to be determined by the extent to which uptake hydrogenases within the same cell recycle the produced hydrogen (Evans et al., 1981). Hydrogen uptake may occur either because (1) nitrogenase-mediated H_2 production

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is energetically wasteful so that H_2 recycling is of physiological benefit to the N_2-fixers (Evans et al., 1981), or (2) hydrogen consumption (requiring oxygen) serves to protect oxygen sensitive nitrogenases in non-heterocystous nitrogen fixers (Saino and Hattori, in press). The degree to which recycling takes place varies widely from organism to organism (Evans et al., 1981), and it seems that each species must be examined to determine its significance as a hydrogen producer. Saino and Hattori (in press) have demonstrated dramatic hydrogen production by *Oscillatoria thiebautii* incubated under argon. However the use of argon instead of nitrogen as an incubation gas may result in enhanced hydrogen production rates, as N_2 is known to compete with protons for electrons from nitrogenase reduction activity (Winters and Burris, 1976). In addition, Conrad and coworkers (1982) found large hydrogen maxima in Lake Loclat, Switzerland, at depths just above a layer of *Oscillatoria rubescens,* although hydrogen concentrations were not found to be correlated with population densities of *O. rubescens,* or of diatoms or algae.

Additional evidence which suggested that hydrogen production by *Oscillatoria* might be important included the reports of ratios of acetylene reduction to nitrogen-fixation (as measured using $^{15}N_2$) which differed markedly from the theoretical ratio of 3:1. The nitrogen fixation reaction can be written as

$$N_2 + 6\,H^+ + 6\,e^- \xrightarrow[\text{nitrogenase}]{} 2\,NH_3$$

while an equivalent amount of reductant shunted to acetylene (ethylene production) can be described by the reaction

$$6\,CH \equiv CH + 6\,H^+ + 6\,e^- \xrightarrow[\text{nitrogenase}]{} 6\,CH_2 = CH_2.$$

Use of the acetylene reduction assay to monitor nitrogen fixation tacitly assumes all the electrons used to reduce acetylene were originally involved in nitrogen fixation, since the presence of acetylene inhibits nitrogen fixation (Rivera-Ortiz and Burris, 1975). Based on the above reactions, the theoretical ratio of ethylene production (acetylene reduction) to nitrogen fixation thus would be 3:1. Measurements of acetylene reduction to nitrogen fixation ratios for *Oscillatoria thiebautii,* however, suggest this ideal situation does not occur. For example Carpenter and Price (1977) reported an average ratio of 6.3:1, and Mague et al. (1974) have reported values ranging from 3.3:1 to 56:1. Since it is known that all nitrogenases mediate production of hydrogen from protons, and since acetylene blocks partially this reaction as well as nitrogen fixation (Burns and Hardy, 1975; Rivera-Ortiz and Burris, 1975), acetylene reduction to $^{15}N_2$ fixation ratios of greater than 3:1 have been interpreted as indicating the presence of significant nitrogenase mediated hydrogen production.

The study described in these pages represents an investigation of the importance of H_2 production by a specific pelagic nitrogen-fixing organism *(Oscillatoria thiebautii)* in maintaining hydrogen supersaturations in a marine environment.

METHODS

Sampling

Two field trips to the West Indies Laboratory of Fairleigh Dickinson University on the eastern end of St. Croix, US Virgin Islands were made from 5 to 19 May and from 8 to 22 December, 1981. Samples were collected from a Boston Whaler in Buck Island Channel (May and December) and north of Buck Island off the shelf edge break in water depths of up to 1000 m (December). General sampling areas are shown in Fig. 1. Because of the very narrow shelf north of St. Croix, even deep water samples could be returned to the shore-based laboratory within 1 to 2 h of collection.

In May, air samples were collected on land from behind the laboratory. In December air samples were collected from the Boston Whaler during return of the boat to the dock, or from the end of the West Indies Laboratory dock during onshore winds. Air samples were collected using plastic disposable syringes fitted with Hamilton valves. Although plastic syringes are permeable to gases, results suggest that this technique is adequate if analysis is prompt. Wind speeds were measured using a hand-held wind meter.

Samples for hydrogen analysis were taken from a Boston Whaler between plankton tows. In May, all samples were taken in ground-glass stoppered glass bottles by leaning over the side of the boat and submerging the bottle beneath the surface. Bottles were held horizontally to permit water to enter the bottles with as nonturbulent a flow as was possible. Sea water which was later filtered and used for incubations was also collected in this manner. In contrast, in December, although a few surface water samples were taken in this manner, most were collected using an eight liter Niskin bottle lowered on a Dacron line weighted at the bottom with a painted weight. In this manner we were able to collect profiles to depths of 50 m. Comparison of samples collected using the Niskin sampler and samples taken directly over the side of the boat indicate that the Niskin bottle contributed no contamination to the water samples. On rough days, shallow samples could only be collected with the Niskin samplers as it was impossible to avoid trapping bubbles in the over-the-side sampling method.

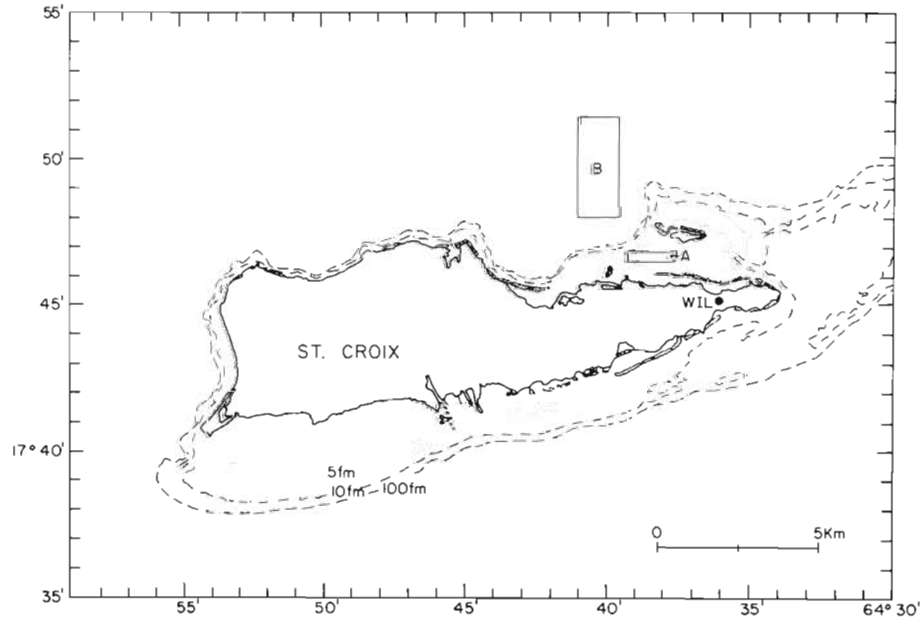

Fig. 1. Locations of sample collection. During May field trip, samples were taken from Area A; during December, from both Areas A and B. Location of the West Indies Laboratory (WIL) indicated by dot

Oscillatoria thiebautii were collected using 0.5 m diameter, 150 micron mesh plankton nets. During the December sampling, these nets were fitted with General Oceanics model 2030R–2 flow meters with the slow speed rotor to permit calculation of the water volume filtered. Plankton tows were generally 15 minutes long at speeds of less than 1 knot. Water volumes filtered ranged from 50 to 200 m^3. Plankton samples for counting were preserved in Lugols-acetate solution (Carpenter, 1971), the tufts (colonies) were disaggregated using a vortex mixer, and individual trichomes were counted in a Sedgewick-Rafter counting chamber.

Hydrogen analyses

Water samples were analyzed for hydrogen as described by Herr et al. (1981) and Scranton et al. (in press) using a vacuum extraction technique to remove dissolved gases from water samples. Analytical precision for replicate water samples was about 15 %.

The standard used was a commercially prepared (Scott) mixture nominally containing 0.95 ppmv hydrogen in nitrogen. Intercalibrations of this standard yielded a hydrogen concentration of 0.99 ppmv for our standard referenced to that used by Texas A&M University (S. Schauffler, pers. comm.). Inaccuracies in hydrogen concentration of a few percent will not affect the discussions below. Absolute calibration and further intercalibrations with Texas A&M, Naval Oceanographic Research and Development Activity (NORDA), and the Naval Research Laboratory (NRL) are underway.

Nitrogen-fixation assays

Acetylene reduction to ethylene was used as an assay for N_2-fixation (Carpenter and McCarthy, 1975). *Oscillatoria thiebautii* were collected as described above, colonies were washed by transfer to filtered seawater using acid washed Pasteur pipettes, and 10 colonies of *O. thiebautii* were transferred using Pasteur pipettes to each of several acid washed 5 ml vials. Water volumes were adjusted to 2.5 ml. The vials were fitted with long-skirted rubber serum stoppers, and 1 ml of acetylene gas generated from calcium carbide was added by syringe. The serum stoppers were pierced with a syringe needle to return the vials to atmospheric pressure. Vials containing filtered seawater with added acetylene but no *Oscillatoria* and vials with 10 colonies of *Oscillatoria* in 2.5 ml filtered seawater with no added acetylene were used as controls. Three or more replicates were run for each assay if sufficient biological material was available. Both experimental and control vials were incubated for various periods (2 h in most cases). In May, the vials were placed in a 1 or 2 layer screen bag (providing about 64 % and 41 % of incident light respectively) and suspended in a glass aquarium flushed with running seawater. In December, vials were placed in 3.8 cm diameter polybutyrate core liners supported at a 45 degree angle to vertical. Placement of 1 or 2 layers of screening over the liners reduced light levels to the samples to 64 % and 41 % of incident light respectively. All incubations were performed under natural sun, usually from noon to 1400 local time. The incident radiation, while not measured directly, was much more intense in May than in December when solar declina-

tion was lower and clouds were more common. When the incubations were complete, vials were returned to the lab and poisoned with 0.2 ml saturated mercuric chloride solution. Ethylene concentrations were measured on a Carle AGC 211 gas chromatograph equipped with a flame ionization detector and a 2 m long, 3.2 mm diameter Poropak R column. Standards used were Scotty II gas mixtures calibrated at MSRC using a Linde custom grade ethylene standard.

Hydrogen production assays

Hydrogen production assays were carried out in much the same way as the acetylene reduction assay, with differences as noted below. To remove hydrogen from the vials initially, headspaces were flushed with hydrogen-free air, nitrogen, or argon for 30–45 s before capping. No differences were observed between samples incubated under air and nitrogen. Flushing was done in such a way as to minimize disturbance to the organisms (Carpenter and Price, 1976). Hydrogen production was assayed by displacing the headspace with water and by injecting essentially all of the headspace into the hydrogen analyzer. Both filtered seawater and killed *Oscillatoria* controls were included in the assay. All data presented represent blank corrected numbers. The operation of the hydrogen analyzer used is based on the mercuric oxide reduction technique as described by Seiler and Schmidt (1974) and as modified by Herr and Barger (1978). Instrumental design was after Herr and Barger (1978). Absolute detection limits for hydrogen were about 0.05 nmole hydrogen in May and about 0.01 nmole hydrogen in December. (The variation in detection limit was due to lower baseline noise in December, as well as to several instrumental modifications made between the two field trips.) Analysis precision for injection of hydrogen standards is about 3 %. Precision of air analyses in both May and December was about 15 %, although previous experience on board oceanographic vessels (Herr et al., 1981; Scranton et al., in press) suggests that at least some of this variability may be due to inhomogeneities in the air near St. Croix. Conservatively, we place our analytical precision at 15 % for these 2 field trips. We attribute larger differences between replicates than this to biological variability.

RESULTS

Atmospheric hydrogen concentrations

Hydrogen was measured in St. Croix air during both December and May 1981. These data are presented in

Table 1. Atmospheric hydrogen concentrations presented as means of replicate samples (parts per million by volume) ± 1 standard deviation. (n): number of replicates

Date	Mean (ppmv)	(n)
May 9	0.44 ± 0.08	(7)
10	0.54 ± 0.09	(6)
11	0.51 ± 0.08	(7)
14	0.55 ± 0.07	(4)
16	0.56 ± 0.08	(4)
Dec 12	0.57 ± 0.04	(2)
15	0.56 ± 0.04	(7)
16	0.61 ± 0.04	(3)
17	0.59 ± 0.05	(3)
19	0.59 ± 0.04	(3)

Table 1. Previous values reported for oceanic air range from 0.55 and 0.58 ppmv (Schmidt, 1978; Schmidt, 1974) to about 0.62 (Herr and Barger, 1978; Herr et al., 1981; Scranton et al., 1982). It appears likely that this difference between the 2 groups represents an interlaboratory calibration difference. An intercalibration between the various U.S. laboratories engaged in hydrogen work is currently underway.

Water samples

Data from the analysis of water samples appears in Table 2 and Fig. 2 for May and December respectively. The principle difference between the results from the 2 field trips is that while the surface waters were systemmatically supersaturated in hydrogen with respect to the atmosphere in May, in December supersaturation was observed only on December 14. All discussions of supersaturation and undersaturation assume an atmospheric concentration of 0.58 ppmv and the solubility equations presented by Wiesenburg and Guinasso (1979).

During December vertical profiles of hydrogen concentrations were made to depths of about 50 m (sampling depth was limited to relatively shallow depths by the use of Niskin bottles on a hand held rope). Fig. 2 demonstrates that hydrogen distributions in December exhibited no maxima on most days, although maxima thinner than 10 to 20 m could have escaped detection. On December 14, the only day on which supersaturated hydrogen concentrations were observed, it appeared that the top 25 m of the water column were enriched in hydrogen. At greater depths, concentrations were similar to those observed on other days. December 14 was a calm, clear day which followed an extended period of southerly winds and generally overcast skies.

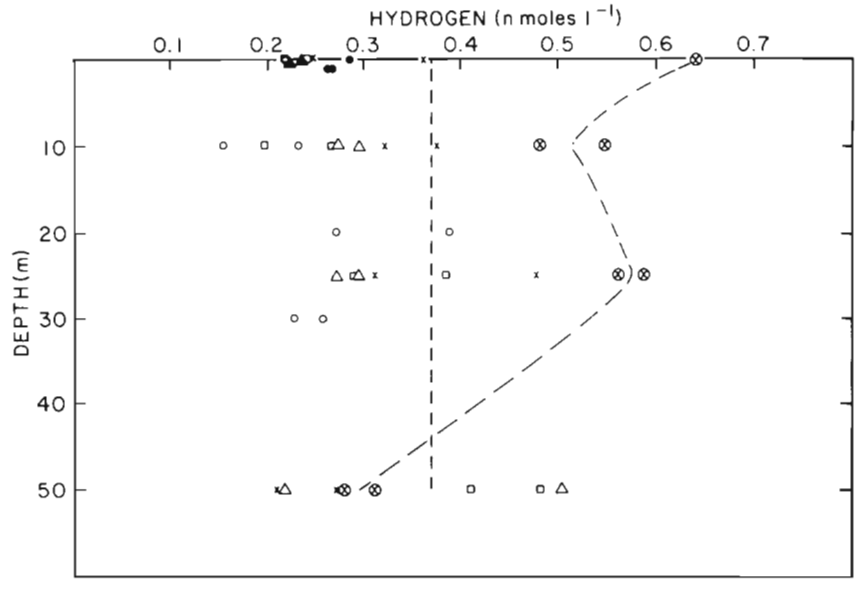

Fig. 2. Hydrogen profiles measured during December 1981 near St. Croix. ● 11 December; ○ 12 December; × 13 December; ⊗ 14 December; △ 16 December; ▲ 17 December; □ 19 December

Oscillatoria cell concentrations

Large day-to-day variations in concentrations of *Oscillatoria* species were observed during both May and December, although quantitative data are available for December only. During our December field trip, cell densities ranged from about 10 cells l^{-1} to more than 2000 cells l^{-1}. All samples were collected in the same geographic region (Fig. 1) so the data are suggestive of major temporal and/or spatial variability in *Oscillatoria* populations. Estimates of the numbers of cells per colony indicate values of about 22 000 cells colony^{-1} were typical (with a standard deviation of about 4000). This agrees well with results from Carpenter and McCarthy (1975) who found values of 29 800 ± 7800 cells colony^{-1}. Since only a few samples were counted to determine the number of cells per colony in this study, the results of Carpenter and McCarthy (1975) have been used in calculations below.

Table 2. Surface water hydrogen concentrations determined in May ± 1 standard deviation. (n): number of replicate samples

Date	Hydrogen concentrations (nmole l^{-1})	(n)
May 8	0.63 ± 0.04	(4)
9	1.02 ± 0.16	(6)
10	1.11 ± 0.08	(5)
11	1.70 ± 0.18	(3)
14	1.18 ± 0.08	(4)
16	1.20 ± 0.09	(3)

Hydrogen production and nitrogen fixation assays

Data for hydrogen production and acetylene reduction are presented in Fig. 3. No conversion has been made between nitrogen fixation and acetylene reduction because no data on $^{15}N_2$ reduction rates are available for our experiments. Carpenter and Price (1977) have presented data indicating that the average acetylene reduction to nitrogen fixation ratio for *Oscillatoria thiebautii* is about 6.3 : 1 although a large range of values was observed. Based on such a conversion fac-

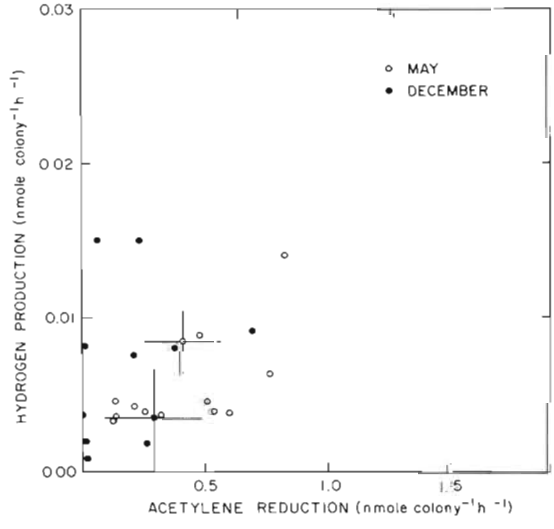

Fig. 3. Plot of hydrogen production rate vs. nitrogen fixation rate for all experiments performed during 2 field trips. Error bars are shown for 2 experiments and indicate typical replicate variability (± 1 standard deviation from mean). No apparent correlation exists between hydrogen production and nitrogen fixation in these experiments

tor and assuming 30 000 cells colony^{-1}, our nitrogen fixation rates range between 0.07 fmole cell^{-1} h^{-1} (2 fg cell^{-1} h^{-1}) and 4.28 fmole cell^{-1} h^{-1} (120 fg cell^{-1} h^{-1}), within the range observed for *Oscillatoria* in other systems (Mague et al., 1974; Carpenter and McCarthy, 1975; Carpenter and Price, 1977; Mague et al., 1977; McCarthy and Carpenter, 1979).

DISCUSSION

The goal of our studies of hydrogen production by *Oscillatoria thiebautii* was to evaluate the importance of this species as an oceanic hydrogen source. Previous results had suggested that hydrogen production might be significant in this species (Saino and Hattori, in press). Since Carpenter and Price (1977) had reported an average acetylene reduction to $^{15}N_2$ fixation ratio of 6.3:1 for this organism, we anticipated hydrogen production exceeding 1 mole H_2 produced for every mole of nitrogen fixed.

Data collected for acetylene reduction and hydrogen production during assays of *Oscillatoria thiebautii* are plotted in Fig. 3. It is clear from these results that no correlation exists between hydrogen production and acetylene reduction. Assuming a 6.3:1 mole ratio for acetylene reduction:nitrogen fixation, hydrogen production to nitrogen fixation ratios range from about 0.04 to about 0.50 moles H_2 produced per mole of nitrogen fixed, substantially lower than predicted from the data of Carpenter and Price (1977). Unfortunately no data are available on $^{15}N_2$ fixation rates for our samples.

Saino and Hattori (in press) have demonstrated that hydrogen is produced in large quantities by *Oscillatoria thiebautii* incubated under argon. We performed a few experiments comparing hydrogen production under argon and under nitrogen and observed that hydrogen production under argon was usually several times higher than under nitrogen. However our hydrogen production rates were still much lower than those observed by Saino and Hattori (in press). Data are not adequate to evaluate this discrepancy but it appears that incubation under nitrogen will more closely imitate natural conditions.

Our conceptual model for determining the importance of *Oscillatoria thiebautii* as an oceanic hydrogen source is based on the observation that, at steady state, any hydrogen flux out of the mixed layer to the atmosphere must be balanced by net hydrogen production within the mixed layer. Thus if we calculate the flux out of the mixed layer using the thin film model as presented by Danckwerts (1970) and Broecker and Peng (1974), we have an estimate of the net hydrogen production within the mixed layer. We can then compare this number to an estimate of hydrogen production within the mixed layer made using our population density data and our data on hydrogen production rates.

Biological consumption or photochemical production or decomposition of hydrogen in the mixed layer represent possible additional terms in the hydrogen budget not included in the simplified model described here. Biological consumption probably is significant as most of our water analyses indicated the surface waters were somewhat undersaturated in hydrogen relative to solubility equilibrium. If this term is large, predicted production rates, required to balance estimated sinks, are low. Unfortunately, no data are available to permit us to evaluate this term. Also, photochemical processes could act as an additional hydrogen source (Herr, pers. comm.) although light intensities were similar on days when supersaturations and undersaturations were observed. Further data on both these processes are required before they can be included in any model.

Quantitative data on *Oscillatoria* densities and on vertical distributions of hydrogen in the water column are only available for December. During December, hydrogen concentrations in the water column were low (Fig. 2), generally at or below atmospheric equilibrium. Therefore during most of this period there was no evidence for net production of hydrogen within the mixed layer.

On 2 dates, however, the water column showed equilibrium and supersaturated hydrogen concentrations. On December 13, the water column was at equilibrium with the atmosphere to depths of about 25 m, while waters at 50 m were slightly undersaturated. On December 14, the water column was significantly supersaturated with respect to atmospheric equilibrium to depths of 25 m, with 50 m concentrations only slightly below saturation. By December 16, the water column had returned to general undersaturation. Thus the hydrogen distributions indicate the occurrence of a net hydrogen production event within the water column, superimposed on persistent hydrogen consumption, probably by hydrogen bacteria (Gest, 1954).

A calculation of the net hydrogen fluxes predicted from the thin film model for air-sea gas exchange (Broecker and Peng, 1974) is presented in Table 3. The thin film model assumes that both the atmosphere and the oceanic mixed layer are well mixed and that transport between the reservoirs is controlled by molecular diffusion through a nonturbulent laminar boundary layer at the air-sea interface.

$$F = \frac{D\,(C_{measured} - C_{equilibrium})}{z}$$

where F = flux of gas across the interface; D =

Table 3. Hydrogen production and flux[a] calculations

Date	Measured[b] C_{H2} (nM)	Equilibrium C_{H2} (nM)	z (μm)	Flux (nmole cm^{-2} s^{-1})	Approximate cell density (cells l^{-1})	Hydrogen[c] production (pmole $colony^{-1}$ h^{-1})	Integrated[d] production (nmole cm^{-2} s^{-1})
May 9	1.02	0.34	15	2.24×10^{-5}		3.9 ± 2.6	
10	1.11	0.34	30	1.27×10^{-5}		4.3 ± 2.8	
14	1.18	0.34	20	2.08×10^{-5}		6.3 ± 2.8	
16	1.20	0.34	20	2.13×10^{-5}		4.5 ± 3.3	
Dec 11	0.27	0.36	20	-2.1×10^{-6}	324	2.0 ± 1.9	1.5×10^{-8}
12	0.25	0.36	15	-3.4×10^{-6}	575	0.8 ± 0.9	1.1×10^{-8}
13	0.32	0.36	50	-4.0×10^{-7}	2200	15.0 ± 17.5	7.6×10^{-7}
14	0.57	0.36	40	2.4×10^{-6}	310	9.0 ± 3.0	6.4×10^{-8}
16	0.31	0.36	15	-1.5×10^{-6}	280	8.0 ± 7.0	5.2×10^{-8}
19	0.27	0.36	50	-8.0×10^{-7}	333	15.0 ± 14.0	1.2×10^{-7}

[a] Flux calculated using $D = 4.96 \times 10^{-5}$ cm^2 s^{-1}
[b] Average of samples collected in mixed layer
[c] Errors indicated are 1 standard deviation for the number of replicates run (usually 3 to 6)
[d] Assumes uniform biological activity over 25 m deep mixed layer

molecular diffusion coefficient in water; $C_{measured}$ = measured gas concentration; $C_{equilibrium}$ = gas concentration predicted from solubility considerations; z = thin film thickness.

The diffusion coefficient for hydrogen in water at 28 °C was estimated from Broecker and Peng (1974). In the calculation a value for the thin film thickness was predicted from wind speeds (Emerson, 1975), average mixed layer hydrogen concentrations were calculated from our vertical hydrogen profiles, and calculated equilibrium hydrogen concentrations were determined using measured temperatures and assuming a uniform salinity value of 36 ‰ S. Uncertainties in salinity values of from 35 to 37 ‰ S would change the equilibrium concentration only 0.1 nmole l^{-1}, well within our analytical uncertainty. On December 13, the water column was a small net sink for hydrogen from the atmosphere, but on December 14, the surface ocean was a significant net source for the gas.

Clearly our assumption of 'steady state' conditions, used in calculation of fluxes from the thin film model, is incorrect. Changes in hydrogen concentrations of the magnitudes observed, on time scales of a day or two indicate the possible importance of processes such as *in-situ* variability in activity of hydrogen producers or consumers, and/or water mass movement through the area bringing in parcels of water with varying biological populations or hydrogen contents. Nevertheless, a qualitative comparison can be made of estimates of total net production determined from air-sea flux calculations and estimates of *in-situ* hydrogen production by *Oscillatoria* made from population density and hydrogen production rate data from the shore based incubations. Estimates of hydrogen production rates from incubation data are presented in Table 3. Production rates for the 13th and 14th are approximately equal, but because *Oscillatoria* densities are so much lower on the 14th, the estimate of water column hydrogen production is a factor of ten lower than on the previous day.

On December 13th, the estimated hydrogen production by *Oscillatoria* is significantly greater than the flux of hydrogen across the air-sea interface. This suggests hydrogen concentrations within the mixed layer should be increasing with time. Indeed, hydrogen concentrations on the 14th are markedly higher than observed the previous day.

It must be noted, however, that the large variations observed in *Oscillatoria* population densities tends to support a hypothesis of significant water parcel movement into and out of the sampling area, or of strong patchiness in the organism's distribution. Therefore, the significant production of hydrogen on the 13th probably cannot be used to quantitatively explain hydrogen excesses on the 14th. However, the calculation of hydrogen production from the 13th indicates that it is possible for *Oscillatoria* populations observed in nature to produce hydrogen at a rate sufficient to maintain hydrogen excesses of the magnitude frequently encountered in the ocean.

In our calculation of hydrogen production we have assumed a uniformly distributed *Oscillatoria* population throughout the top 25 m of the water column. With our sampling technique, we were unable to determine vertical distributions of *Oscillatoria* populations. However Carpenter and Price (1977) and McCarthy and Carpenter (1979), among others, have shown that the abundance of these organisms can change markedly

with depth. In a number of the stations occupied by Carpenter and Price in the Caribbean Sea, cell concentrations varied by almost a factor of 10 within the top 25 m, with maximum concentrations observed at around 15 m. It is possible that our plankton tows, usually at depths of 5 to 10 m significantly undersampled the populations present. Thus we feel that the hydrogen production calculations present in Table 2 are probably a minimum estimate and indicate that it is possible that *Oscillatoria* may represent a significant hydrogen source for the mixed layer. Our general observation in December that, except for 14 December, hydrogen concentrations were low when population densities were low is compatible with this hypothesis.

Unfortunately, during May when the water was consistently supersaturated with hydrogen, we have no quantitative data to permit us to calculate population densities. Our qualitative impression was that *Oscillatoria* colonies were considerably less abundant in December than in May. During May we also have no data on the depth to which hydrogen excesses extended. Using observed production rates per colony and the thin film model, we estimate that we would have required colony densities of about 4 l^{-1} to maintain the entire mixed layer excess, if it extended to depths of 25 m as in December. Colony densities were probably substantially less than this, so we feel that in May, either H_2 production by *Oscillatoria* represented only a fraction of total net hydrogen production in the mixed layer, or that excess hydrogen concentrations were confined to a smaller fraction of the water column than was the case in December. All May samples were taken in Buck Island channel which has a water depth of between 10 and 20 m.

There are also uncertainties associated with the use of hydrogen production rates measured in vials containing 10 colonies in 2.5 ml seawater. Data from May indicated that acetylene reduction rates were constant over the 2 h incubation period and rates on a per-colony-per-hour basis also proved to be relatively constant regardless of the number (5 to 20) of colonies used. However, hydrogen production rates were sufficiently variable that linearity over the 2 h incubation could not be confirmed, and hydrogen production per colony seemed to depend somewhat on colony number. One explanation for this observation is that the hydrogen producing and/or consuming mechanism within the cells is highly sensitive to ambient hydrogen concentrations. The amounts of hydrogen produced within the vials during the incubations represents 2 orders of magnitude more hydrogen than was present initally in the 2.5 ml water phase. If equilibration with the headspace is slow during the incubations (a possibility since the vials are not shaken or disturbed) the incubating organisms may be exposed to unusually high hydrogen levels, perhaps stimulating their hydrogen uptake systems. If this is the case, our production rates represent lower limits to the true hydrogen production. In future experiments, this hypothesis can be tested using large volumes of water for the incubations.

One possible additional source for hydrogen production in the May samples that would be less important in the December samples taken offshore could be hydrogen production on or near the coral reefs north of St. Croix. In May, our samples were taken in Buck Island channel which is quite near the reefs along the north side of St. Croix and near Buck Island (Fig. 1). In addition, numerous sea grass beds are found in patches throughout the channel. Nitrogen fixation is known to be high in both coral reef and tropical sea grass environments (Capone, in press), so our samples may have contained hydrogen produced by these systems as well. However, it should be noted that several samples taken in Buck Island channel on December 11 yielded low hydrogen concentrations similar to those seen further offshore.

CONCLUSION

Our results suggest that a complex relationship exists between hydrogen production by *Oscillatoria thiebautii* as determined by short-term assays and hydrogen concentrations within the oceanic mixed layer. Nevertheless, we have demonstrated some important phenomena: that *O. thiebautii* can produce hydrogen in significant quantities when incubated under hydrogen-free nitrogen or air; that in some cases this production rate is sufficient to permit development of elevated hydrogen concentrations in the mixed layer; that short-term spatial and temporal variability in hydrogen concentrations and in *O. thiebautii* population densities and activity may be significant in the hydrogen balance of the mixed layer. Further work will investigate the effect of crowding in observed hydrogen production rates.

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