# On the relationship between hydrogen saturation in the tropical Atlantic Ocean and nitrogen fixation by the symbiotic diazotroph UCYN-A

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Key points: Hydrogen supersaturation widespread across a tropical N. Atlantic transect.

Saturations correlated with UCYNA *nifH* abundance

High resolution $H_2$ measurements are capable of illustrating space and time scales of UCYN-A diazotrophy

Headings:

## Abstract

Dissolved hydrogen measurements were made at high resolution in surface waters along a tropical north Atlantic transect between Guadeloupe and Cape Verde in 2015 (Meteor 116). Parallel water samples acquired to assess the relative abundance of the *nifH* gene from several types of diazotrophs, indicated that *Trichodesmium* and UCYN-A were dominant in this region. We show that a high degree of correlation exists between the hydrogen saturations and UCYN-*A nifH* abundance, and a weak correlation with *Trichodesmium*. The findings suggest that nitrogen fixation by UCYN-A is a major contributor to hydrogen supersaturations in this region of the ocean. The ratio of hydrogen released to nitrogen fixed has not been determined for this symbiont, but the indications are that it may be high in comparison with the small number of diazotrophs for which the ratio has been measured in laboratory cultures. We speculate that this would be consistent with the diazotroph being an exosymbiont on its haptophyte host. Our high resolution measurements of hydrogen concentrations are capable of illustrating the time and space scales of inferred activity of diazotrophs in near real-time in a way that cannot be achieved by biological sampling and rate measurements requiring incubations with $^{15}N_2$. Direct measurement of high resolution spatial variability would be relatively challenging through collection and analysis of biological samples by qPCR, and extremely challenging by $^{15}N$-uptake techniques, neither of which methods yields real-time data. Nonetheless, determination of fixation rates still firmly depends on the established procedure of incubations in the presence of $^{15}N_2$.


## 1 Introduction

It has long been known that nitrogen fixation, the critical process for maintaining a source of combined nitrogen to biota, involves production of molecular hydrogen. Pioneering work on the relationship between hydrogen concentrations and nitrogen fixation in the oceans was reported in a series of papers by Herr (e.g. *Herr and Barger, 1978*) and Scranton (e.g. *Scranton et al. 1982*). *Ogo et al*. [2004] propose that the $H_2$ is displaced from a dihydride-activated reaction centre of the nitrogenase enzyme. While the stoichiometry of the Equation 1 indicates

$$N_2 + 8H^+ + 16ATP + 8e \rightarrow 2NH_3 + H_2 + 16\ ADP \qquad \text{Equation 1}$$

displacement of one hydrogen molecule for each $N_2$ reduced, the few laboratory studies made on marine diazotrophs show highly variable net releases, with a value of 0.28 reported for *Trichodesmium*, one order of magnitude less for *Cyanothece*, and two orders of magnitude less for *Crocosphaera* [*Wilson et al., 2010*]. The lower than stoichiometric releases are attributed to recycling of hydrogen by its producer. We propose that diazotrophy in the oceans may (depending on the extent to which the main diazotrophs release rather than recycle hydrogen) lead to an easily measured supersaturation of hydrogen capable of acting as a general indicator of nitrogen fixation. This is made possible by the fact that hydrogen concentrations in the atmosphere are reasonably uniform in each hemisphere with mixing ratios of *ca*. 0.5 ppm in the northern hemisphere, and 0.52 ppm in the southern [*Simmonds et al., 2000*]. These concentrations reflect the balance of major sources of hydrogen, namely biomass burning and photolytic oxidation of hydrocarbons such as methane and isoprene in the atmosphere [*Levy, 1972; Novelli et al., 1999*], and sinks – oxidation by OH in the atmosphere, and bacterial

consumption in soils which is weighted towards the northern hemisphere [*Levy, 1972; Rhee et al., 2005*]. Coupled with the low aqueous solubility of hydrogen, the low atmospheric abundance leads to a low and easily calculated equilibrium concentration in surface waters. Against this background any net source of $H_2$ in nitrogen fixation should show as a significant supersaturation [*Moore et al., 2009*]. Processes acting to diminish any saturation include loss to the atmosphere, consumption by other microorganisms in the water column [*Punshon et al., 2007; Barz et al., 2010*], and downward mixing.

Nitrogen fixation rates can be quantified using $^{15}N_2$ incubation methods and subsequent isotope ratio mass-spectrometric analysis [*Montoya et al., 1996*]. This method, however, is labour-intensive and unless attention is paid to complete equilibration of the tracer, prone to errors such that nitrogen fixation by diazotrophs is underestimated [*Großkopf et al., 2012; Mohr et al., 2010*], and batches of $^{15}N_2$ need to be checked for$^{15}NH_3$ contamination [*Dabundo et al., 2014*]. Analysis of dissolved $H_2$ on the other hand can be carried out by a semi-automated system at sea with measurements every 3.5 minutes [*Moore et al., 2014*]. While it cannot provide a measure of nitrogen fixation rates, particularly because of the complexities introduced by unquantified biological consumption rates, as well as the unknown degree of recycling by diazotrophs themselves, it has been proposed as a near real-time indicator of active nitrogen fixation [*Moore et al., 2009*] with the potential to survey large areas of the ocean for active nitrogen fixation and to support $^{15}N_2$ incubation studies by identifying important areas for accurate rate determinations. The objective of the work presented here is to further explore the relationship between $H_2$ saturations and nitrogen fixation by diazotrophs, using qPCR to enumerate several *nifH* phylotypes in DNA and RNA samples. The qPCR measurements of the *nifH* phylotypes, expressed as *nifH* DNA copies $L^{-1}$ provided a measure of the distribution and relative abundance of the major diazotrophs known to inhabit oceanic waters within this region of the Tropical Atlantic. Although *nifH* RNA levels are indicative of active nitrogen fixation, they are out of phase with protein synthesis, and therefore do not usually directly correlate with nitrogenase [*Church et al., 2005*]. It should be noted that for logistical reasons during the cruise described here direct measurements of nitrogen-fixation, for example by $^{15}N_2$ uptake, could not be performed. In addition, any such incubation measurement that requires long incubation periods (24-48 h in oligotrophic waters) cannot yield results that are congruent with the high resolution $H_2$ data.

## 2 Methods

Dissolved $H_2$ concentrations were measured at 3.5 minute intervals in the surface ocean during research cruise M116 on board RV Meteor (1$^{st}$ May-3$^{rd}$ June 2015, Pointe-à-Pitre, Guadeloupe, to Mindelo, Cape Verde, Figure 1).

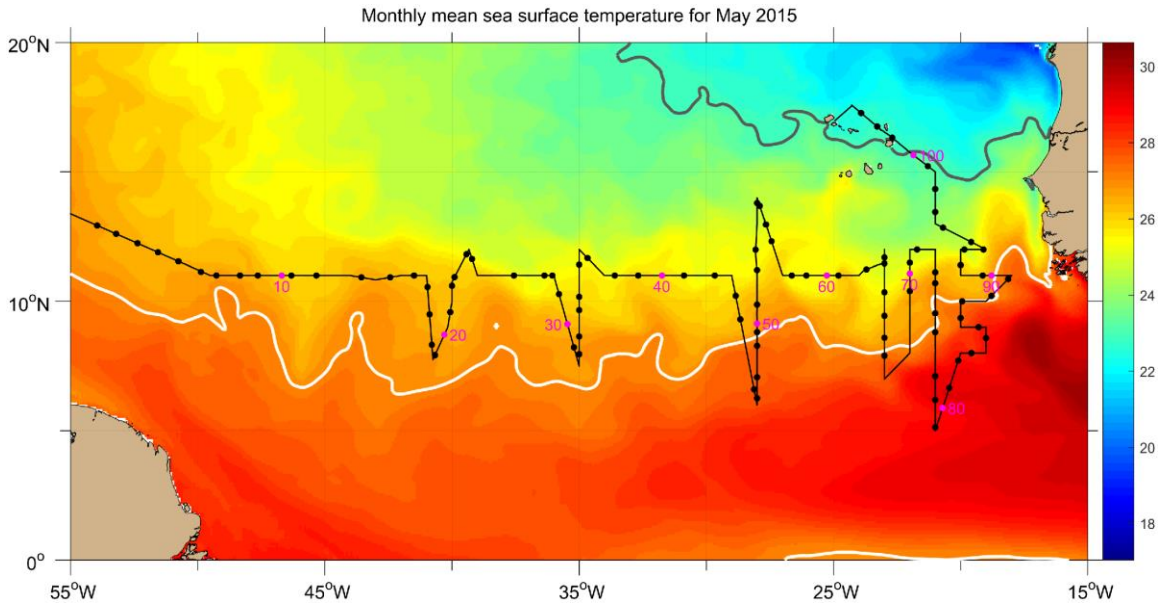


**Figure 1.** Cruise track and monthly mean sea surface temperature (SST) for May 2015. The month's average SST was calculated from the daily SST predictions from an operational forecast system (PSY4V3R1, see text for details). The ship track is shown by the black line, and dots denote positions of 103 discrete nucleic acid samples; sample numbers are indicated at intervals of 10. The 27°C isotherm is shown by the white contour, and 23°C by grey.

## 3 Physical Oceanographic Conditions

An overview of the prevailing physical oceanographic conditions of the study region was obtained from an operational global data-assimilative ocean 1/12° physics analysis and forecast system (PSY4V3R1). The PSY4V3R1 uses the NEMO 3.1 (Nucleus for European Models of the Ocean) modelling system with a horizontal resolution of 9 km at the equator and 50 levels in the vertical with 1m resolution near the surface. We focused on daily mean fields of sea level, sea surface temperature, salinity and current. To assess the reliability of model predictions of sea surface temperature and salinity we compared them with the corresponding observations made from the ship. The standard deviation of the differences was 0.39°C and 0.15 for temperature and salinity, respectively.

The general circulation in the shiptrack-covered equatorial region is briefly reviewed. Previous studies have shown that the zonal mean surface circulation, from 20°N towards the equator includes (1) the westward flowing North Equatorial Current (NEC), (2) the seasonal eastward flowing North Equatorial Countercurrent (NECC), and (3) the westward flowing Northern South Equatorial Current (NSEC, see Philander, 2001; Talley *et al*., 2011). The NECC lies between 3°N and 10°N and begins to form at about 5°N in May, in response to a northward shift of the Intertropical Convergence Zone (ITCZ) associated with heavy rainfall. These features are evident in the mean surface circulation predicted by PSY4V3R1 for the study period. In addition, relatively low surface salinity was observed adjacent to the southernmost stations (locations of Samples #46 and #79 in Figure 1) in the ITCZ in May 2015 that, based on the atmospheric fields used to drive the ocean forecast system, was due to precipitation associated with the passage of an intense storm. Two coastal currents are also evident in the monthly mean SST field shown in Figure 1: the North Brazil Current and the Canary Current. The North Brazil

Current carries warm water of South Atlantic origin to the northwest along the coast of Brazil. The Canary Current is associated with relatively cold upwelled water, and flows along the African coast from north to south.

## 4 Hydrogen analysis

The analytical method is described in *Moore et al.* [2014]. In brief, water from the ship's clean seawater supply was introduced by Tygon tubing to the bottom of a glass reservoir and allowed to overflow. A peristaltic pump (REGLO Digital, Ismatec) supplied seawater from the bottom of the reservoir to a bubble-segmented glass coil equilibrator [*Moore et al., 2014; Xie et al., 2001*], with hydrocarbon-free air (Ultra Zero Air, Praxair) being used to provide the gas bubbles in the equilibrator, as well as a carrier gas for the $H_2$ analyser. The gas phase was separated from the water phase at the top end of the equilibrator and fed into a 1 mL sample loop of a Peak Performer 1 Reducing Compound Photometer (PP1 RCP, Peak Laboratories, LLC). $H_2$ and CO were separated on a molecular sieve column and their concentrations were measured using a heated mercuric oxide bed and UV absorption detector. The instrument's built-in software was used to evaluate peak areas. $H_2$ measurements were corrected with measurements of a low concentration gas standard (1.135 ppm) after every 20 seawater measurements. Furthermore, equilibrator efficiency was monitored daily throughout the cruise using seawater equilibrated with a 4.93 ppm $H_2$ standard (Praxair). The low concentration standard was prepared by gravimetric dilution of the 4.93 ppm standard in zero air. Seawater concentrations of $H_2$ were calculated using equilibrium solubilities described by *Wiesenburg and Guinasso* [1979].

Zinc anodes on the ship's hull can lead to $H_2$ contamination, while biofilms growing inside the seawater plumbing can take up $H_2$ and lead to an underestimation of concentrations in seawater. As the seawater intake was at the bow of the ship, contamination from anodes during transit was highly unlikely; the plumbing was PVC and thus not a potential $H_2$ source. Therefore, only data for ship's velocities above 6 knots were used for analysis. Another potential problem is the accumulation of biofilms within seawater pipes. Bacteria forming those films can consume $H_2$ [*Moore et al., 2009*], so the seawater pipes leading to the laboratory were cleaned with diluted hypochlorite bleach (12%) before the cruise and again on 23 May 2015 as an originally planned precaution against regrowth of biological films. To monitor for $H_2$ consumption within the seawater pipes, water was collected from the thermosalinograph inlet, approximately 6 m downstream of the bow inlet and compared with underway measurements. Underway and discrete samples were usually within 0.2 nmol $L^{-1}$.

## 5 Filtered seawater sample collection and nucleic acid extraction

Water samples were collected for filtration and qPCR analysis, typically three times a day during transit from the same laboratory seawater used for $H_2$ measurements, giving a total of 103 samples. Approximately 3L of seawater were collected in a low-density 4 L polyethylene bottle. A 10 ml disposable pipette with an attached prefilter 160 µm mesh was connected to Masterflex tubing and lowered into the sample collection bottle and the seawater sample was filtered onto a 3 µm filter, followed by a 0.2 µm filter (both Isopore, Millipore) using a peristaltic pump at 30 rpm (FH100 Peristaltic Variable Pump System, Thermo Scientific). The filtration was stopped after 20 min to minimize degradation of RNA, and the exact filtration volume was recorded. The 3 and 0.2 µm filters were placed in cryotubes and flash-frozen in liquid $N_2$. Samples were stored at -80°C until analysis. We considered the sum of the two filters for all of

the measurements. The 160 µm mesh was used to prevent copepods and other large zooplankton from overwhelming the microbial community signal with DNA from multicellular eukaryotic organisms. The use of a pre-filter may have led to an underestimation of large *Trichodesmium* filaments and colonies when present. However, the density of *Trichodesmium* colonies reported in this region range from 0-2900 colonies $m^{-3}$ and is on average at a density of less than 500 colonies $m^{-3}$: Singh *et al*. 2017), making it unlikely that they would have been present in a 3 L water sample. Furthermore, the prefilter did not prevent the collection of the trichomes (*Trichodesmium* filaments) as these were visually observed on the 3 µm filters in several areas that are known habitat for Trichodesmium. Samples were extracted using the AllPrep DNA/RNA Mini Kit (Qiagen) following manufacturer's instructions. RNA was transcribed to cDNA using SuperScript III Reverse Transcriptase (Invitrogen) and PCR primers *nifH*2 and *nifH*3 (Zehr *et al*., 2003).

## 6 qPCR

Abundances of nifH gene or transcript copies per liter of seawater were estimated for filamentous cyanobacteria (*Trichodesmium* and *Katagnymene* – hereafter referred to as simply *Trichodesmium*), UCYN-A (*Candidatus Atelocyanobacterium thalassa*), UCYN-B (*Crocosphaera*), UCYN-C (*Cyanothece*), *Rhizosolenia* (*Richelia* symbionts, H1), *Hemiaulus* (*Richelia* symbiont, H2), Cluster III and the γ-proteobacterial group Gamma A, using nifH-phylotype specific primers and TaqMan probes and following the method described in Langlois *et al*. (2008). Environmental DNA and cDNA samples were diluted 1:5 with qPCR water and 5 µL was added to the qPCR reaction. Samples were measured on a ViiA 7 Real-Time PCR thermocycler (Applied Biosystems) and analysed using the manufacturer's software. The default cycling program was used but the number of cycle was increased from 40 to 45. The Taqman assays were calibrated using nucleotide standard specific for each *nifH* phylotypes as described in Langlois *et al*., [2008]. The qPCR results are reported in *nifH* copies $L^{-1}$ for the various phylotypes and this measurement cannot be extrapolated to cell counts and cannot be intercompared quantitatively. In particular, it has been recently established that *Trichodesmium* is polyploid, i.e. it contains several copies of the genome within a cell, making the *nifH* copies $L^{-1}$ much higher than the cell density (Sargent *et al.,* 2017). Therefore, the *nifH* assays are used here only to assess the relative abundance within a specific phylotype. The various phylotypes estimates cannot be added together to provide a total estimate of the total *nifH* copies $L^{-1}$ as a proxy for diazotroph abundance.

## 7 Results

Hydrogen saturations, shown in Figure 2, were highly variable but normally greater than 100% with a maximum of ca. 850%, and averaging around 250% (0.8 nmol $L^{-1}$). The only significant period with substantial undersaturation was for 13 hours at 23°W, 9-11°N (May 22 2015) when saturation averaged 68%. No significant correlations were observed between saturation and sea surface temperature, salinity, global radiation, windspeed (u, or $u^2$), or time of day.

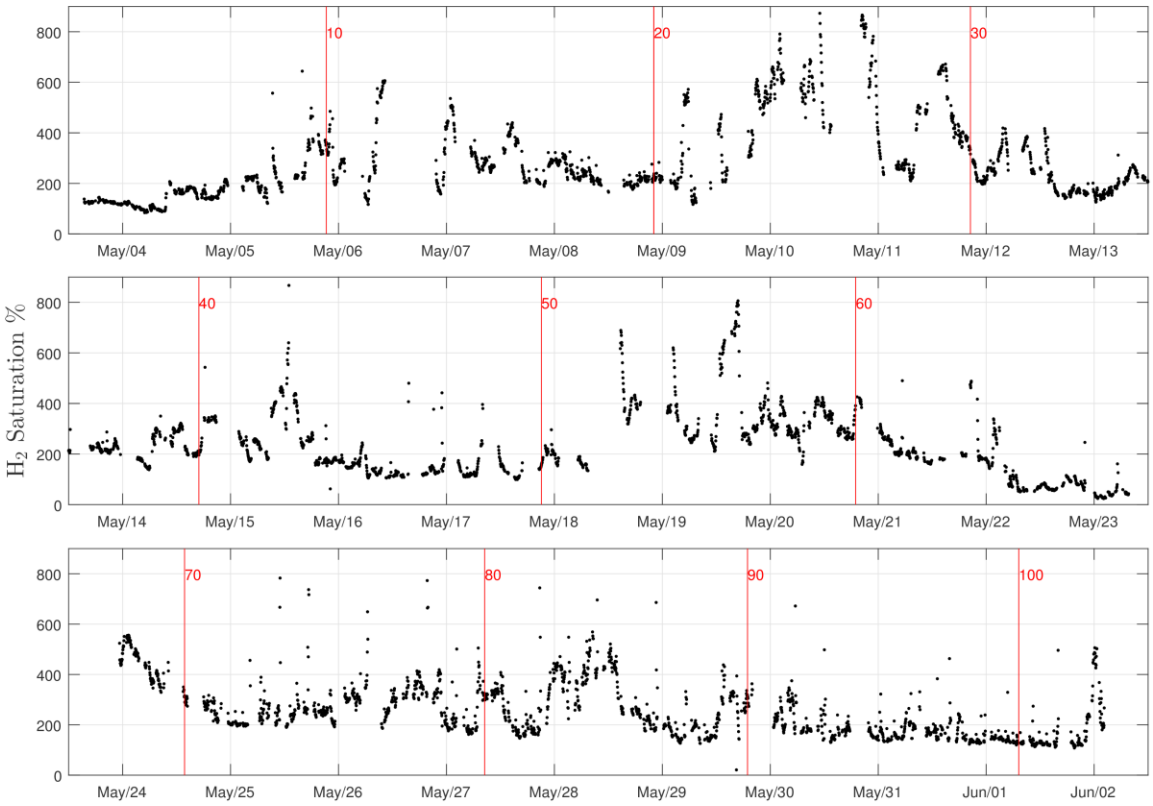


**Figure 2.** Hydrogen saturations along the cruise track plotted against time. The vertical lines indicate the sampling time of stations marked in Figure 1.

As noted in the Method section, the absolute values of Taqman assays (*nifH* DNA copies $L^{-1}$) for the various diazotrophs targeted here are neither representative of the cell density nor the cellular biomass. The results were used in this study to assess the distribution and relative abundance of each *nifH* phylotype in relation to the $H_2$ saturation. Throughout the transect, *Trichodesmium* and UCYN-A were the most widely distributed diazotroph groups targeted by the qPCR assays in the surface waters of the tropical Atlantic. The other nifH phylotypes (see supplementary data) measured here were detected more sporadically throughout the transect and showed no correlation with $H_2$ saturation and these groups are not further treated here. The Taqman assays made with cDNA, representative of nifH transcript levels, also indicated that *Trichodesmium* and UCYN-A were actively transcribing the *nifH* gene.

*Trichodesmium nifH* DNA concentrations (Figure 3) were generally high and variable to the west of 28°W (Samples 0-51), and mostly low to the east with the exception of some high and variable values between 20 and 21°W (Samples 84-90). UCYN-A was most abundant between 48 and 23°W (Samples 7-67, Figure 3) with the abundance showing clear signs of being very low or undetected in surface waters warmer than about 27°C (compare panels (b) and (d)). These warmer waters also supported relatively high abundances of *Trichodesmium* (compare panels (c) and (d)). A single high value of UCYN-A occurred in the coastal waters off Cape Verde (24°W; sample #103). A correspondence is apparent between samples having the highest abundance of UCYN-A *nif*H copies and those with the highest $H_2$ saturations (Figure 3). This is examined in more detail below.

A number of factors point to the existence of two groupings of samples, those west of 23°W (Samples 1-64), and those to the east. It is possible that it is related to an increase in phosphate that occurs east of 23°W (Figure 3e; note the log scale of phosphate). Further comments on differences between these regions are given in the Discussion. In the following treatment we look first at the relationship between $H_2$ saturations and UCYN-A nifH abundance in the sample western group (Samples 1-64) which cover most of the ocean basin.

Figure 4 suggests a linear relationship between the log of the $H_2$ saturation and the log of UCYN-A abundance. In contrast there is no correlation between the $H_2$ saturation of these samples and their abundance of *Trichodesmium* (Figure 5). However, the same figure shows that if we select just those samples having UCYN-A abundances in the lowest one third of the entire group, then some correlation emerges. It appears that the influence of *Trichodesmium* on $H_2$ saturation is overwhelmed by the influence of UCYN-A.

Using the relationship derived from Figure 4 a prediction (Figure 6) can be made for $H_2$ saturations based solely on UCYN-A abundance in this western zone of the cruise track. Inspection of this plot suggests the predictive capability of a single variable, the abundance of a single species of diazotroph, is remarkable, particularly in view of the fact that a realistic model of hydrogen concentration would demand inclusion of loss to the atmosphere and microbial consumption, the rates of which are unknown and presumably strongly dependent on the composition of the local microbial community which itself may be affected by the hydrogen concentration.

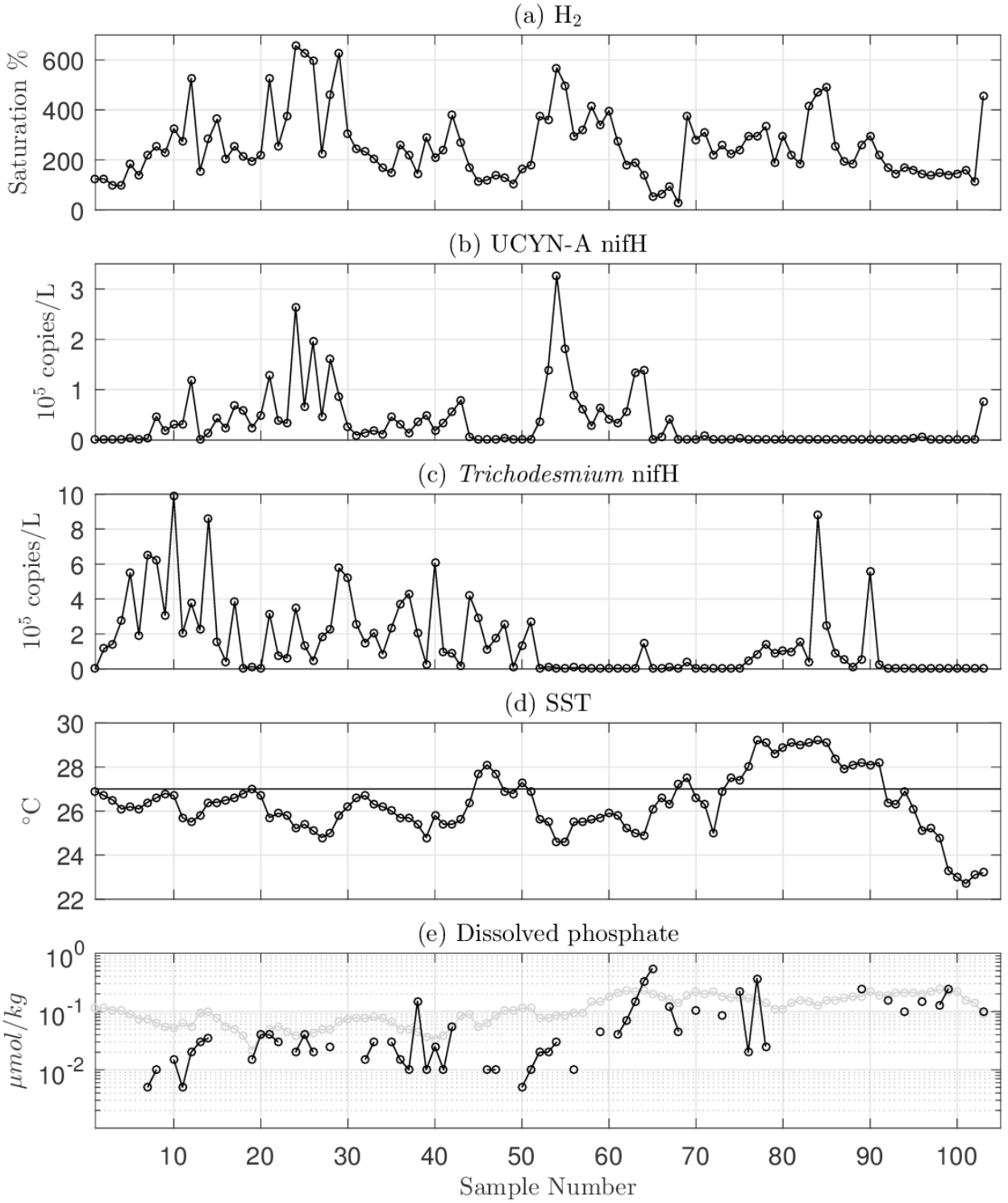


**Figure 3.** (a) Hydrogen saturations (average of 3 continuous measurements over the time of discrete water sample collection), (b) UCYN-A abundances, (c) *Trichodesmium* abundances, and (d) SST for discrete samples collected along the cruise track where horizontal line denotes T=27°C, the temperature maximum for UCYN-A; (e) measured dissolved phosphate

concentration (black circles) and mean surface phosphate based on interpolating the monthly mean World Ocean Atlas 2013 version 2 climatology (Garcia *et al*., 2014) for May to the ship track (grey line). Refer to Fig.1 for exact sample locations.

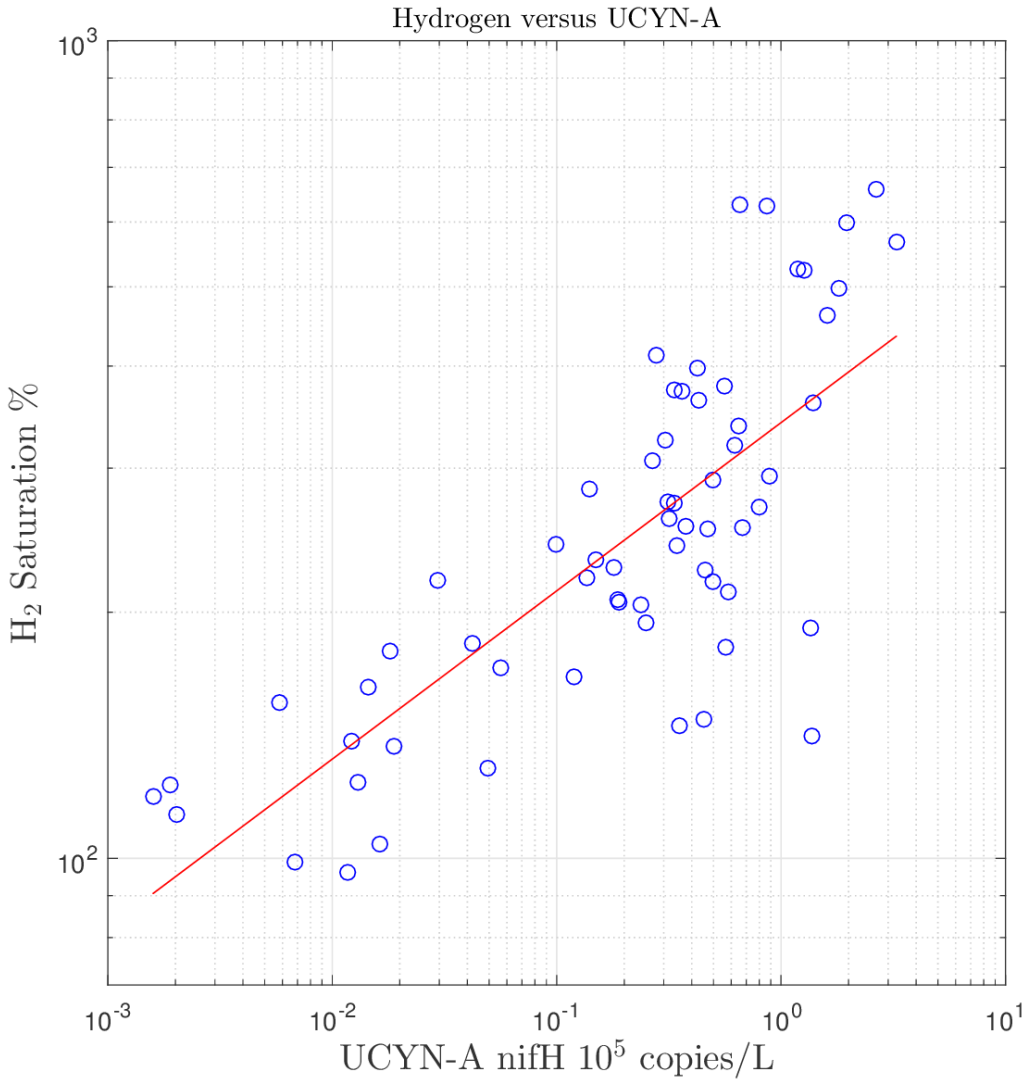


**Figure 4.** Log-log plot of $H_2$ saturation against UCYN-A *nifH* copy abundance for Samples 1 to 64 inclusive. The red line shows the linear regression of $\log_e(H_2)$ on $\log_e$(UCYN-A). The intercept, slope and coefficient of determination were estimated to be 5.83±0.10, 0.206±0.030 and $R^2$=0.588±0.102. The standard errors were estimated using the circular bootstrap with blocking to allow for serial correlation of $\log_e(H_2)$ on $\log_e$(UCYN-A). A block length of 8 was selected based on simulation studies using a bivariate AR(1) process matched to the observations.

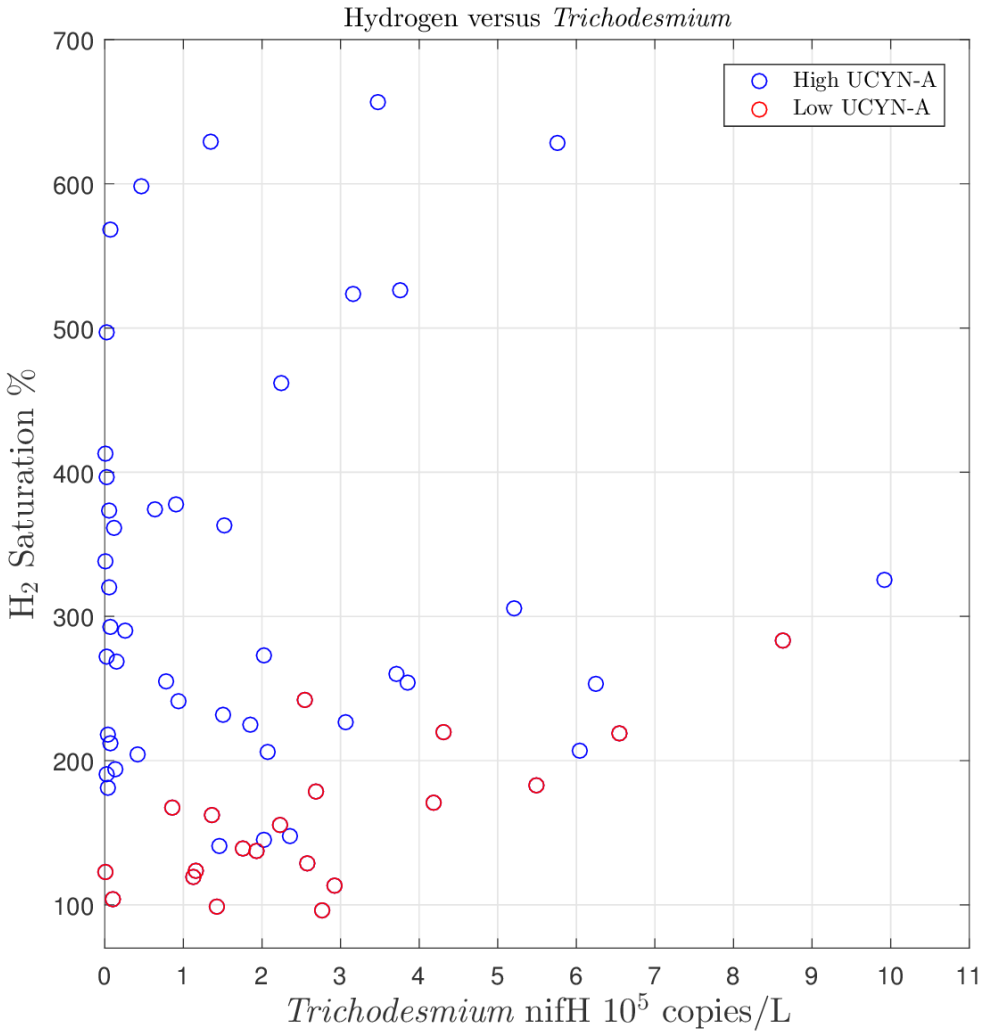


**Figure 5**. $H_2$ saturation plotted against Trichodesmium *nifH* gene abundance for 64 western samples; in red are the samples with low UCYN-A abundance ("low" defined in text).


**Figure 6.** $H_2$ saturation plotted (log scale) against sample number; red symbols represent observations, and blue represent predicted saturations based on UCYN-A *nifH* copy abundance.

## 8 Discussion

Hydrogen supersaturation in ocean surface waters has been reported in many publications [e.g. *Herr and Barger, 1978; Scranton et al. 1982; Moore et al., 2009, 2014*], but in early work there was much uncertainty about the sources, and it was recognised that contamination, particularly from corroding metal, was difficult to avoid [*Scranton et al., 1982*]. It was recognised that nitrogen fixation and photochemistry were potential sources in surface waters [*Scranton 1983, 1984; Herr et al., 1984*]. As the current work focuses on surface waters we will not discuss hydrogen production in anoxic environments, nor hydrothermal sources. *Punshon and Moore* [2008a] have shown that while photochemical production is a large source in highly coloured terrestrial waters, and possibly a source in coastal waters influenced by terrestrial runoff, it is not expected to be significant in offshore ocean waters.

In the same way that nitrogen fixation studies have, over a long period, focussed on the highly visible colonial species, *Trichodesmium*, so too has this species been the subject of studies and discussion of its contributions to hydrogen supersaturation. There have been reports supporting the notion that it produces hydrogen both in field studies [*Scranton 1984; Scranton et al., 1987*], and laboratory studies [*Wilson et al., 2010; Punshon and Moore, 2008b*]. Indeed, with so few quantifications of release ratios of $H_2$ to $N_2$ consumption, as well as very incomplete knowledge of the extent and variety of different marine diazotrophs, it has been natural to link hydrogen supersaturation to *Trichodesmium* activity or abundance. However, *Scranton et al.*

[1982] commented that in the surface waters of the Mediterranean "and probably elsewhere", organisms existing in oxygenated waters other than *Trichodesmium* (then *Oscillatoria*) must be producing hydrogen.

UCYN-A (*Athelocyanobacterium thalassa*) has in recent years been recognised as a potentially important contributor to marine nitrogen fixation [*Zehr el al., 2008; Krupke et al. 2013; Martinez-Perez et al., 2016*], and the current work reveals that it plays a major role in supporting hydrogen supersaturation in the Equatorial Atlantic. The cyanobacterium UCYN-A, lacking the photosystem II and the ability to fix carbon, is dependent on a photosynthetic host that is a haptophyte [*Thompson et al., 2012*]. The absence of oxygen production by this symbiotic diazotroph might facilitate its ability to fix nitrogen [*Bothe et al., 2010*]. The strong correlation shown here between hydrogen saturation and UCYN-A abundance does not itself prove causality, but since we know from Equation 1 that nitrogen fixation yields hydrogen, and since a number of laboratory and field studies provide evidence for this, at least in the case of *Trichodesmium*, we can deduce that nitrogen fixation by UCYN-A is a major, and probably the primary, source of hydrogen saturation in our study. *Martinez-Perez et al*. [2016] report that UCYN-A and its hosts have growth rates five to ten times higher than *Trichodesmium* and that this leads to the conclusion that its contribution to nitrogen fixation is proportionately higher than its cell abundances alone would suggest, those abundances being controlled by active grazing. Stoichiometrically, hydrogen release follows the rate of nitrogen fixation, so the hydrogen signal is not diminished by the grazing that checks the cell abundance. However, we have no information on the rates at which hydrogen is being consumed by bacteria, nor do we know the extent to which the symbionts themselves might recycle hydrogen. It may be quite significant that there is evidence for UCYN-A being an exosymbiont [*Martinez-Perez et al., 2016*], as hydrogen that it must produce may be released directly to the environment, as opposed to being channelled to (or through) its host as in the case of endosymbiotic associations like *Richelia-Hemiaulus*.

In support of the strong correlation that we observed between $H_2$ supersaturation and UCYN-A, we note that the spatial separation between $O_2$ evolution in the host and the symbiotic diazotrophs might provide favourable conditions for diazotrophy and the associated $H_2$ production by the nitrogenase enzyme. In other cyanobacterial diazotrophs, the deactivation of PSII (Bayro-kaiser and Nelson, 2016) and/or the diel cycle segregation of photosynthesis and nitrogen fixation between light and dark periods (Bandyopadhyay *et al.* 2013) may lead to high rates of $H_2$ production under aerobic conditions, by deriving the required energy for nitrogen fixation from glycogen pools, either provided as an additional organic carbon source or acquired through photosynthesis during the day. In the symbiotic UCYN-A, photosynthetically-derived organic carbon from the host is transferred to the symbiont and likely fuels nitrogen fixation (Martinez-Perez *et al.*, 2016). Additionally, the symbiont has the ability to carry out ATP synthesis from sunlight through PSI driven cyclic electron flow (Zehr *et al.* 2016; Bandyopadhyay *et al.*, 2010, 2011; Martinez-Perez *et al.*, 2016).

Diel cycling between nitrogen fixation (at night) and photosynthesis (during the day) is common in unicellular cyanobacteria, but in cyanobacteria that have spatial separation of these two processes, as for heterocystous cyanobacteria, the temporal segregation of nitrogen fixation and photosynthesis is not observed. Except for the fact that we now know that the UCYN-A is a

symbiont on a haptophyte (Thompson *et al.* 2012 and Martinez-Perez *et al*. 2016), its lifestyle is practically unknown because there is no cultured isolate of the symbiont and its host. Therefore, the mechanism by which the symbiont's nitrogenase is protected from oxidative damage by the host is currently unknown.

The reason for the existence of many samples to the east of 23°W that contain low or zero abundances of *Trichodesmium* and UCYN-A is unknown, except that many of the samples are from waters warmer than 27°C (Figure 1) and this appears to be outside the range for UCYN-A. Preliminary analysis of *nifH* sequences obtained by high throughput sequencing also suggest that another clade of UCYN-A and an alphaproteobacterial diazotroph dominated the diazotrophic community east of 23°W (Jenni-Marie Ratten, Julie LaRoche, personal communication), and were not targeted by the qPCR assays used in this study.

Our data show that surface waters can be substantially supersaturated even when UCYN-A and *Trichodesmium* have low abundance. We can speculate on reasons for this: first, the supersaturation might be accounted for by small contributions from several different diazotrophs, including ones that are present at relatively low abundances; second, there may be active diazotrophs other than those targeted by our work; and third, though unlikely, the hydrogen signal sometimes dissipates more slowly than the responsible diazotroph(s).

The strong correlation found in this study leads us to propose that UCYN-A has a significant value for the ratio, $H_2$ release/$N_2$ fixed, almost certainly higher than the values reported for the unicellular cyanobacteria *Cyanothece and Crocosphaera* (only 0.05 and 0.004 mol of $H_2$ per mol $N_2$ fixed), and probably higher than the value (0.28) for *Trichodesmium* [*Wilson et al., 2010*]. It would not be surprising if hydrogen release rates from a very small number of laboratory studies with cultures differ from what occurs in the oceanic environment.

The weak relationship found in this study between hydrogen saturation and *Trichodesmium* abundance, at least in the presence of significant abundance of UCYN-A, may suggest that hydrogen saturations reported in the Pacific [*Moore et al., 2009*] and Atlantic [*Moore et al., 2012*] are indicative of widespread nitrogen fixation by UCYN-A. One question among many yet to be addressed on biological consumption of hydrogen is whether an organism like *Trichodesmium* that is reported to recycle the greater part of the hydrogen it produces would, in waters enriched in the gas from other sources, become a net consumer.

This work in the tropical Atlantic shows for the first time that hydrogen saturations in surface waters can be related to the abundance of UCYN-A (and by inference its diazotrophic activity). Our high resolution measurements of hydrogen (Figure 2) are capable of illustrating the space and time scales of diazotrophy, in this instance apparently attributable to UCYN-A activity. Direct measurement of such variability would be relatively challenging through collection and analysis of biological samples by qPCR, and extremely challenging by $^{15}N$-uptake techniques, neither of which methods yields real-time data. Nonetheless, rigorous determination of nitrogen fixation rates depends on the established procedure of incubations in the presence of $^{15}N_2$.

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**Figure 1.**

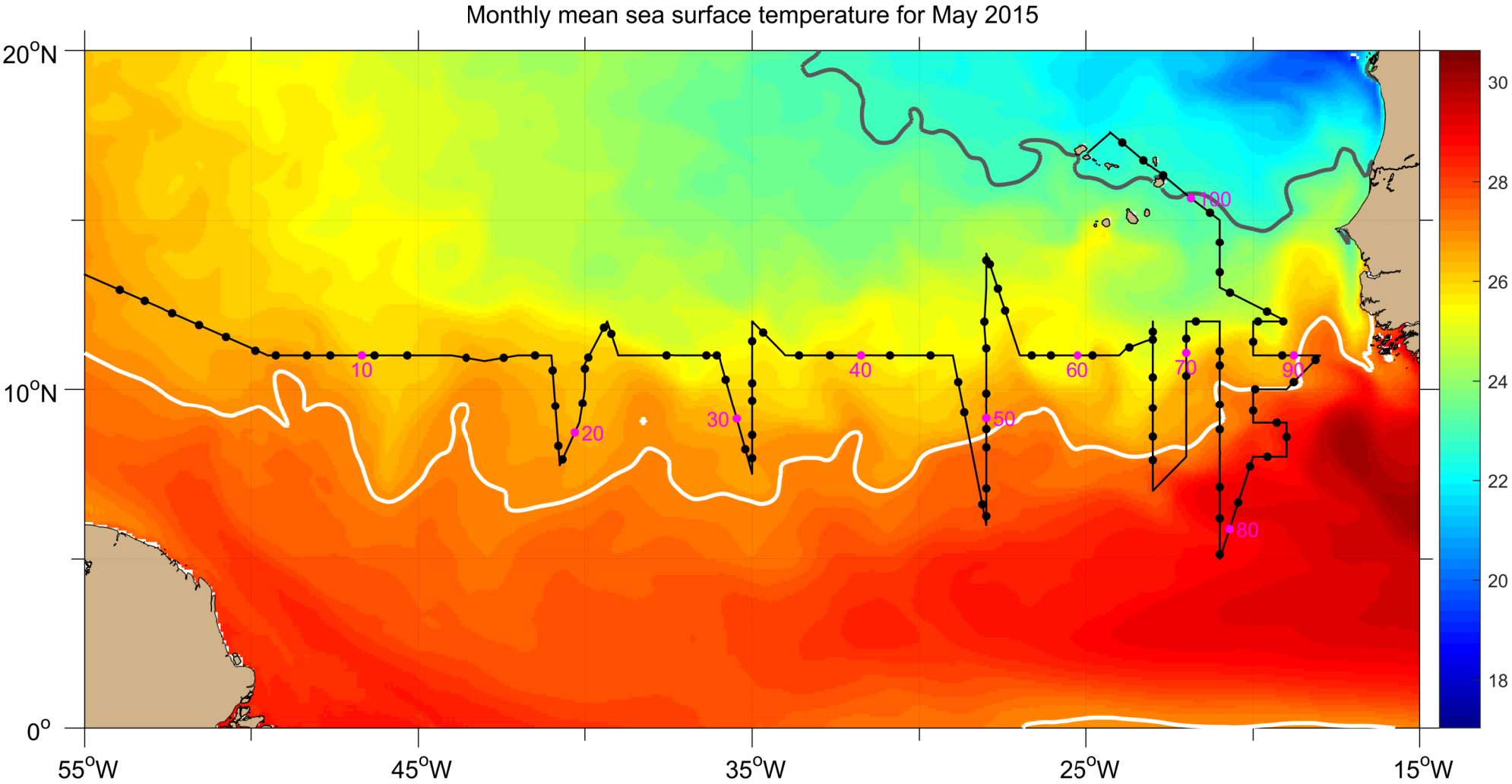

Monthly mean sea surface temperature for May 2015
20°N
10°N
0°
55°W
45°W
35°W
25°W
15°W
10
20
30
40
50
60
70
80
90
100
30
28
26
24
22
20
18

**Figure 2.**

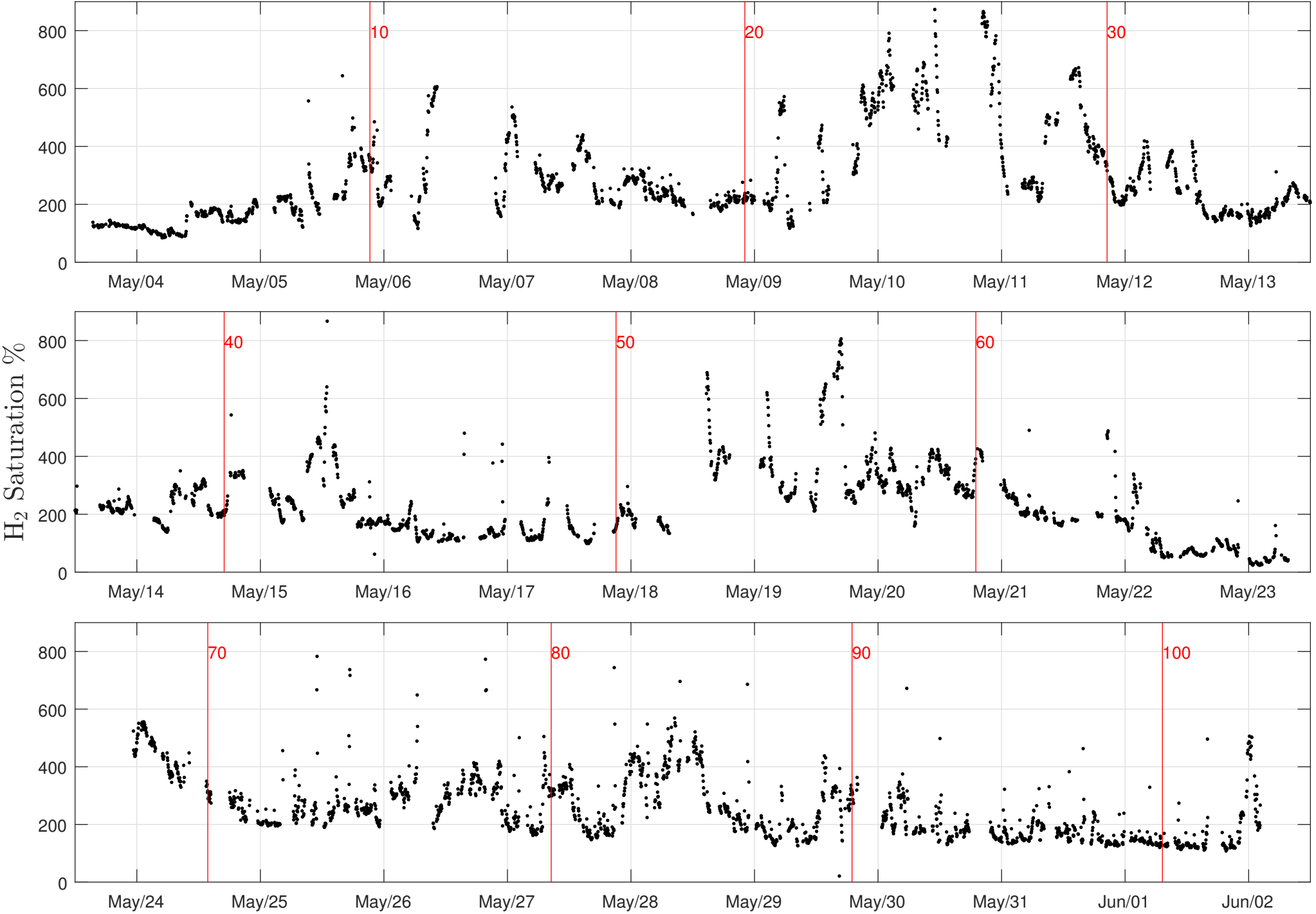

$H_2$ Saturation %
0
200
400
600
800
10
20
30
40
50
60
70
80
90
100
May/04
May/05
May/06
May/07
May/08
May/09
May/10
May/11
May/12
May/13
May/14
May/15
May/16
May/17
May/18
May/19
May/20
May/21
May/22
May/23
May/24
May/25
May/26
May/27
May/28
May/29
May/30
May/31
Jun/01
Jun/02

**Figure 3.**

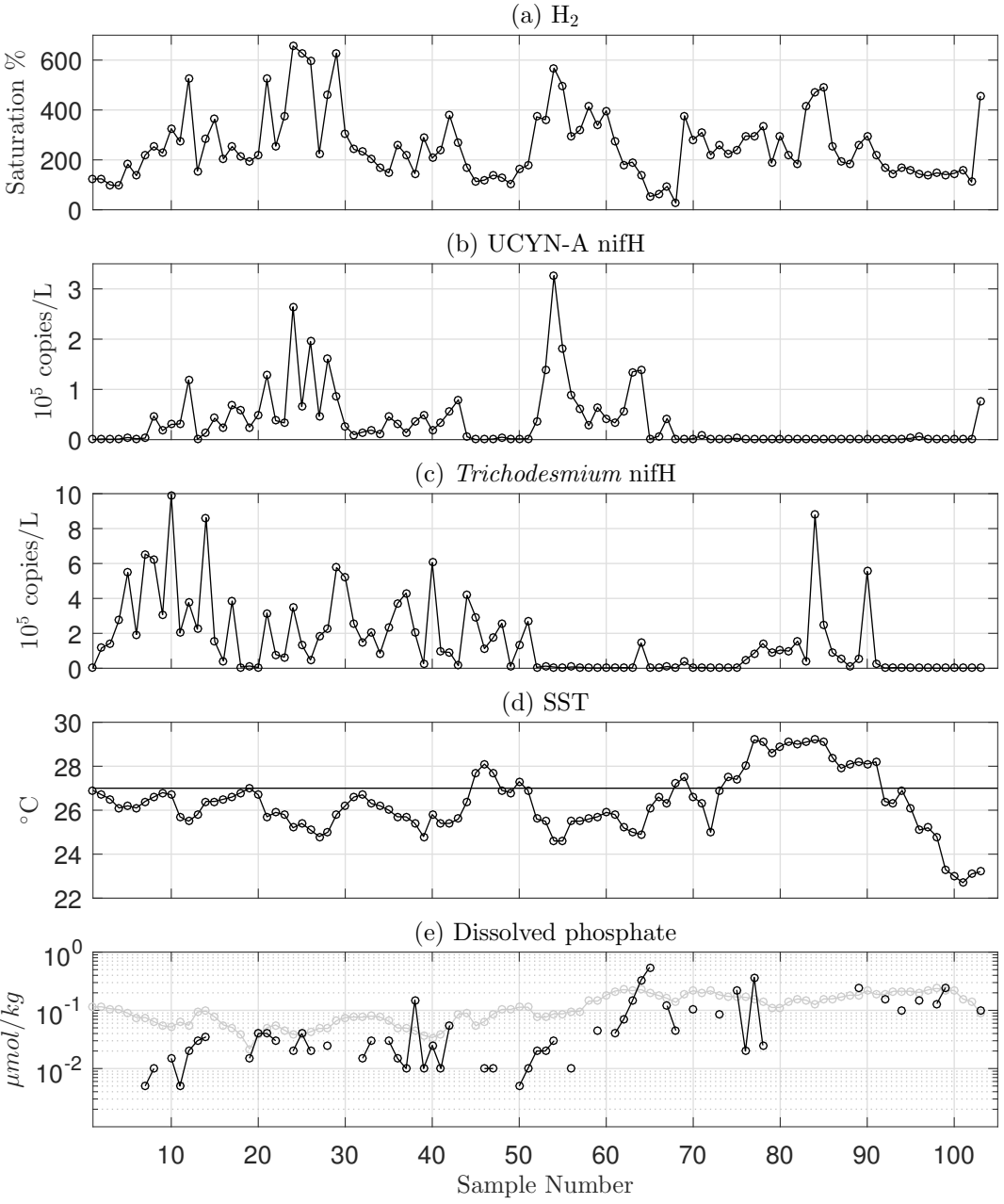

(a) $H_2$
Saturation %
600
400
200
0
(b) UCYN-A nifH
$10^5$ copies/L
3
2
1
0
(c) *Trichodesmium* nifH
$10^5$ copies/L
10
8
6
4
2
0
(d) SST
°C
30
28
26
24
22
(e) Dissolved phosphate
$\mu mol/kg$
$10^0$
$10^{-1}$
$10^{-2}$
10
20
30
40
50
60
70
80
90
100
Sample Number

**Figure 4.**

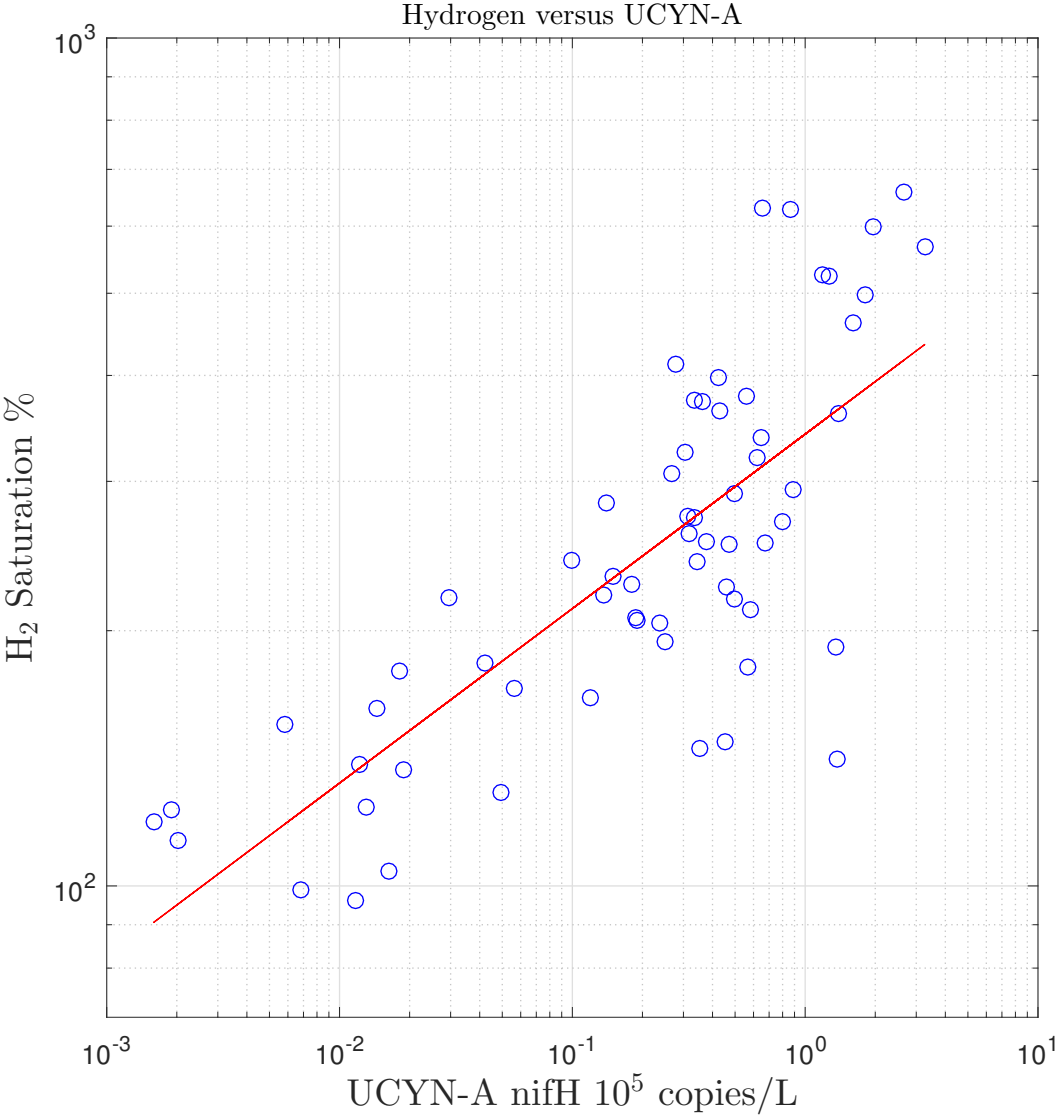

Hydrogen versus UCYN-A
$10^3$
$10^2$
$H_2$ Saturation %
$10^{-3}$
$10^{-2}$
$10^{-1}$
$10^0$
$10^1$
UCYN-A nifH $10^5$ copies/L

**Figure 5.**

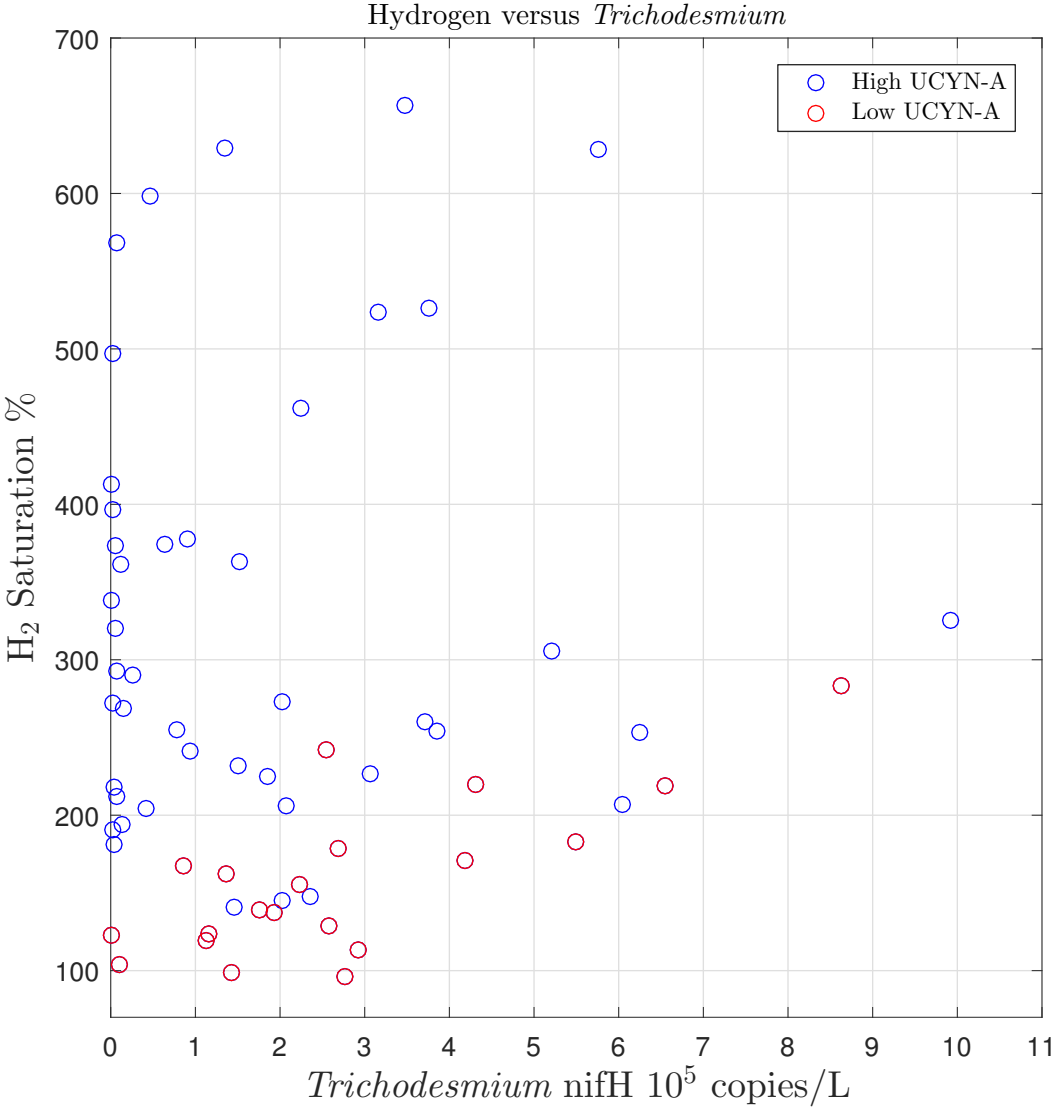
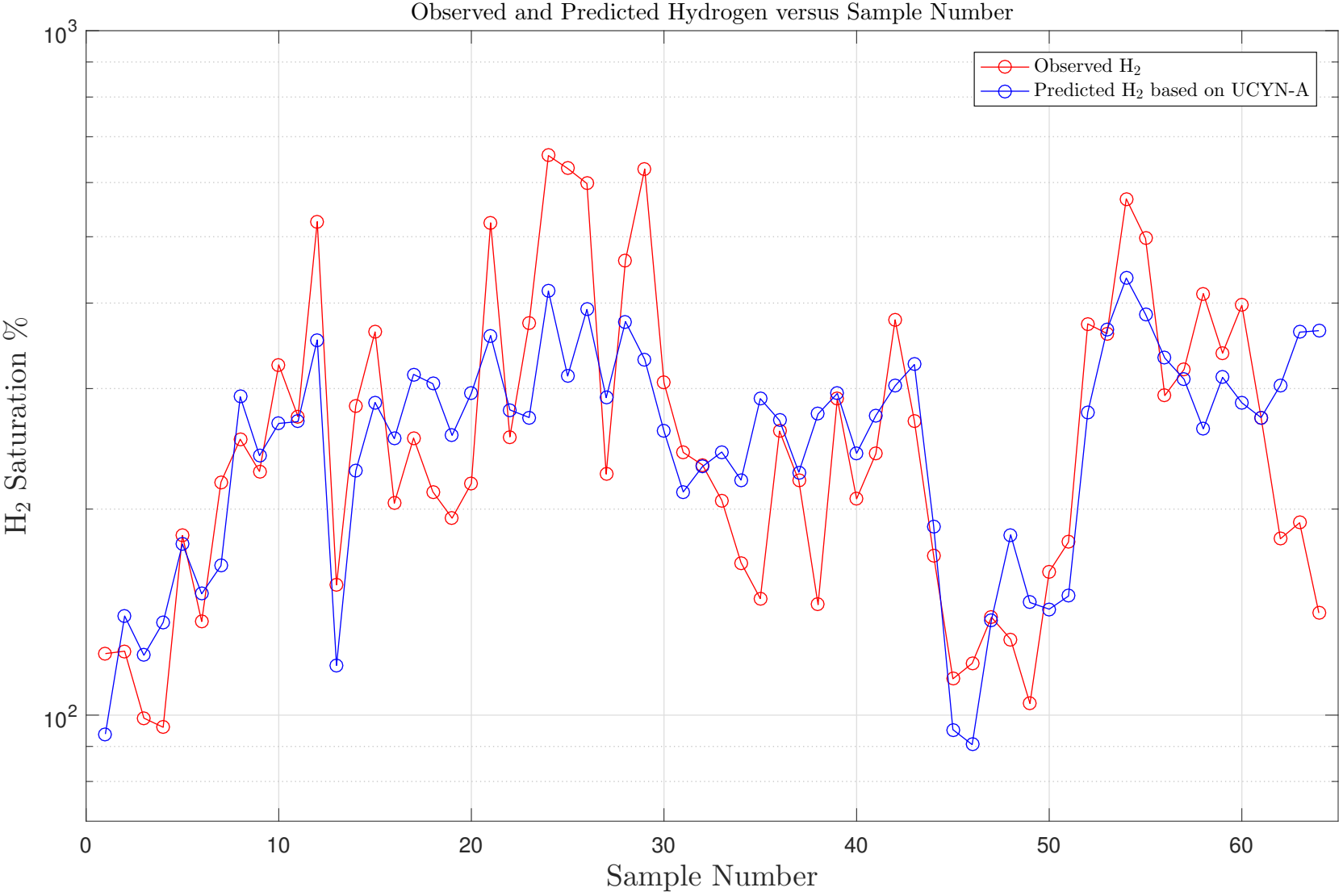

Hydrogen versus *Trichodesmium*
High UCYN-A
Low UCYN-A
$H_2$ Saturation %
*Trichodesmium* nifH $10^5$ copies/L
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11

**Figure 6.**


Observed and Predicted Hydrogen versus Sample Number
Observed H2
Predicted H2 based on UCYN-A
H2 Saturation %
Sample Number
10^3
10^2
0
10
20
30
40
50
60