Volatile Organic Compound Production in *Synechococcus* WH8102

by
Duncan Ocel

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Chemistry and Botany
(Honors Scholar)

Presented May 21, 2018
Commencement June 2018

AN ABSTRACT OF THE THESIS OF

Duncan Ocel for the degree of Honors Baccalaureate of Science in Chemistry and Botany presented on May 21, 2018. Title: Volatile Organic Compound Production in *Synechococcus* WH8102.

Abstract approved:_____

Kimberly Halsey

High-resolution mass spectrometry was used to measure a range of volatile organic compounds (VOCs) in real time as they were produced by the ubiquitous marine cyanobacterium *Synechococcus* WH8102 during a 24-hour light/dark cycle. Ethenone, acetaldehyde, ethanol, isoprene, acetic acid, dimethyl sulfide (DMS), acetone, phenol, and several as-yet unidentified compounds were measured in higher concentration in live cultures than in azide-killed cultures or sterile artificial seawater. Several compounds were found in higher concentration in the daylight part of the diel cycle than in the night, suggesting VOCs are produced during active photosynthesis.

Key Words: phytoplankton, volatile organic compounds, *Synechococcus*, acetaldehyde, dimethyl sulfide

Corresponding e-mail address: oceld@oregonstate.edu

Volatile Organic Compound Production in *Synechococcus* WH8102

by
Duncan Ocel

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Chemistry and Botany
(Honors Scholar)

Presented May 21, 2018
Commencement June 2018

Honors Baccalaureate of Science in Chemistry and Botany project of Duncan Ocel presented on May 21, 2018.

APPROVED:

_____

Kimberly Halsey, Mentor, representing Department of Microbiology

_____

Stephen Giovannoni, Committee Member, representing Department of Microbiology

_____

Cleo Davie-Martin, Committee Member, representing Department of Microbiology

_____

Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

_____

Duncan Ocel, Author

Volatile Organic Carbon Production in *Synechococcus* WH8102

Duncan Ocel

1 February 2018

Introduction:

Synechococcus is a marine cyanobacterium, found ubiquitously in tropical to temperate waters of the euphotic zone (Waterbury et al., 1986). As primary producers, *Synechococcus* take in CO_2 and convert it to sugars and other organic matter. They act as a gateway for carbon flow from CO_2 into the remainder of the marine food web. As such, *Synechococcus* plays a significant role in the carbon cycle at the interface between the ocean and the atmosphere.

Synechococcus strain WH8102 is "representative of *Synechococcus* populations found in oligotrophic areas of the ocean" (Six et al., 2004). This strain has been extensively characterized—the whole genome was sequenced by 2004, and, because it is relatively easy to culture and test in the laboratory, many studies have been published on the properties expressed by *Synechococcus* WH8102 in response to different environmental conditions.

The exchange of volatile organic compounds (VOCs) between the ocean and the atmosphere is a phenomenon that has important implications for climate science, ecology, and oceanography. Little is known about the roles of marine organisms in VOC cycling. Most existing knowledge regarding marine VOC production has emerged from the atmospheric science perspective, but biological factors influencing VOCs are not understood (Zindler, 2013). Some phytoplankton are known to produce VOCs, and some bacterioplankton are known to consume them, but little is known about rates of VOC production and consumption in seawater or by individual plankton species (Halsey et al., 2017; de Bruyn et al., 2017; Schlundt et al., 2017). In the few cases where interspecific interactions have been examined, tight coupling between rates of production and consumption is suggested to exist, but it is unknown to what extent this trend manifests in the global oceans (Halsey et al., 2017). In cases where rates of VOC production and consumption are nearly equal, VOCs are unlikely to escape to the atmosphere. In cases lacking tight coupling, though, VOCs could be an important mechanism of carbon exchange between the ocean and the atmosphere, and other nutrients could also be involved. Methane thiol, for example, is a VOC used as a sulfur source by some of the most abundant bacterioplankton in the ocean (Sun et al., 2016).

Research into the roles of phytoplankton species and interspecies interactions in marine VOC production is needed to fully understand the extent and nature of production/consumption coupling and the extent to which marine VOCs may escape to the atmosphere. VOCs are known to affect hydroxyl radical formation in the upper atmosphere, and ocean-produced VOCs are suspected to reach the upper atmosphere in relevant amounts (Zindler, 2013; Schlundt et al., 2017). Exploring the types and quantities of VOCs produced in the ocean will allow for a better understanding of nutrient cycling, VOC cycling, and atmospheric composition. Furthermore, VOC cycling can influence euphotic zone productivity because VOCs that escape to the atmosphere represent loss of photosynthetically fixed carbon. Thus, VOC dynamics may have

important roles in regulating climate change and ocean acidification (Schlundt et al., 2017; Kanakidou et al., 2005).

Acetaldehyde is known to be produced by the diatom *Thalassiosira pseudonana*. Halsey et al. (2017) reported that acetaldehyde was produced at rates four to eight times faster than two other VOCs, isoprene and acetone. Acetaldehyde contains only carbon, hydrogen, and oxygen, and can be converted to acetyl-CoA catalyzed by aldehyde dehydrogenase and acyl-CoA synthetase and acetyl-CoA can then be fed into lipogenesis or the Citric Acid cycle (Halsey et al., 2017). This simple pathway is known in many bacteria, including *Pelagibacter ubique*, a ubiquitous bacterioplankton (Halsey et al., 2017). Thus, mechanisms exist in bacterioplankton for both acetaldehyde catabolism and its assimilation into biomass.

In addition to acetaldehyde, isoprene and acetone were also produced by *T. pseudonana* (Halsey et al., 2017). Isoprene accounts for more than 50% of biogenic, non-methane atmospheric hydrocarbons and is suspected to be a universal product of phytoplankton (Zindler, 2013). Acetone is important in the creation of peroxyacetylnitrate in the atmosphere, which allows for the transport of bioavailable nitrogen across long distances (Zindler, 2013). These, among other VOCs, are good starting points for analysis of production in other species.

VOCs are difficult to measure in aqueous media because their volatility causes them to be present in low or undetectable concentrations. Recently, a method was introduced to measure VOCs in planktonic suspensions that uses bubbling of cell cultures to force VOCs into the gas phase, which is immediately directed into a proton-transfer-reaction, high-resolution mass spectrometer (PTR-MS) for VOC detection. The proton-transfer reaction is a "soft ionization" method, which results in little to no fragmentation of analytes. Therefore, each isotopic form of each chemical manifests as a single peak, which is useful for instantaneous measurement of complicated samples, like seawater.

The goal of this study was to assess the range of VOCs produced by *Synechococcus* WH8102. It was hypothesized that *Synechococcus* would produce several VOCs in amounts large enough to escape aqueous solution, and that production of some VOCs would align with periods of photosynthetic activity (i.e. VOCs would be produced in the light), as was measured in *T. pseudonana* by Halsey et al. (2017). This hypothesis was tested by measuring the range of VOCs produced by live *Synechococcus* WH8102 cultures and comparing this VOC profile with azide-killed *Synechococcus* WH8102 cultures. This work demonstrates that *Synechococcus* WH8102 produces a wide range of VOCs during photosynthesis. Future work will further our understanding of the network of marine species and VOCs involved in VOC cycling.

Experimental:

Synechococcus WH8102 was grown in four separate 200 mL cultures in 250 mL polycarbonate flasks. Each flask was inoculated from a single starting culture into 190 mL f/2 + Si artificial seawater medium. In anticipation of this experiment, the starting culture was kept in semi-continuous batch culture where ten-fold dilutions were done every 3 or 4 days. Flasks were kept at 18 ºC on a 16-hour light (day) / 8-hour dark (night) cycle. During the 16-hour day,

photosynthetically active radiation (PAR) was constant at 50 µmol photons m^{-2} s^{-1}. During the night, PAR was 0 µmol photons m^{-2} s^{-1}. Cells grew at a specific growth rate of approximately 0.5 d^{-1}. Cultures were maintained axenically with periodic inspection by bright-field microscopy and flow cytometry (FACScan, Becton-Dickinson Immunocytometry Systems, Mountain View, CA).

Cultures in the four flasks grew for three days and were harvested during late exponential phase of growth. Cultures were combined into a single suspension and then 5 x 100 mL were aliquoted into 200 mL test chambers for VOC analysis. Three triplicate test chambers were used for VOC profile measurements. One test chamber was filled with 100 mL sterile f/2 + Si medium, and a fifth chamber was filled with 100 mL of killed cells. These cells were killed by addition of 1 mM sodium azide. The killed culture was gently swirled by hand for a few seconds, and then incubated at room temperature for 60 minutes before the commencement of VOC measurements. Culture transferred to the final (sixth) chamber was used for supporting measurements (chlorophyll, cell density, and carbon and nitrogen analysis).

Test chambers were placed inside a light and temperature-controlled incubator (Bridgepath Scientific, Frederick, MD, United States) set at 30 µmol photons m^{-2} s^{-1} during day hours and 0 µmol photons m^{-2} s^{-1} during night hours (as described above) and 18 ºC. Sterile air was bubbled through one chamber at a time, for 5 minutes, at 50 mL/min, through a glass frit at the base of the chamber (~400 ppm CO2). Air in the headspace was fed directly into the PTR-MS (PTR-TOF 1000, Ionicon Analitik GmbH, Innsbruck, Austria), where the constituent gasses were chemically ionized and accelerated through a drift chamber. Time of flight was measured and used to calculate mass over charge (m/z) ratios. VOC analysis began at 10:10 am (on November 3rd, 2017), which was 3 hours and 10 minutes after the start of the daytime portion of the light/dark cycle. VOC measurement was repeated every 2 hours until cessation of measurement after the last chamber was measured at 10:40 am the following day.

Identification was performed primarily based on the exact mass of the peak. Determination of isomeric identity—that is, the arrangement of atoms in a molecule—of chemicals measured in this way is impossible; in cases with only one isomer, or large Gibbs' free energy differences between isomers, the identity was assumed to be the lowest-energy, most common form. For example, ethenone, $H_2C{=}C{=}O$, can also exist as ethynol, $HC{\equiv}COH$. Ethynol and ethenone readily interconvert, and the concentration of ethenone at standard temperature and pressure (STP) is several orders of magnitude greater than the concentration of ethynol, so this peak was identified as ethenone. In cases where no easy distinction between isomers could be made, no identification was done, or, in the case of phenol, a merely presumptive identification was made.

While bubbling, chemicals in solution which are at least somewhat volatile should depart with approximately first-order kinetics, because, at equilibrium, the concentration in solution is proportional to the concentration in air. Deviations from this pattern occur under some circumstances. When a chemical is very insoluble in air and present in high concentrations in solution, its loss should be described by a zeroth-order function; losses do not vary with

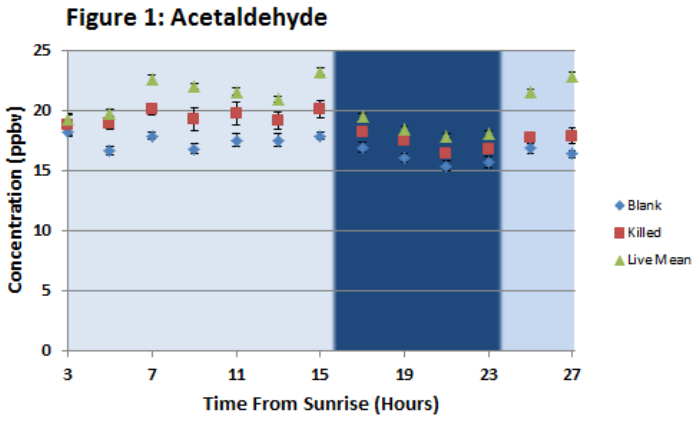
concentration in solution because the chemical is saturated in the air. This set of circumstances was not the case for any of the compounds of interest in this study. Another deviation is as follows: chemicals which are present at very low concentrations in solution and which are very volatile may leave essentially immediately after production, which would mean that instead of having first-order kinetics in a period of constant bubbling, this chemical would leave solution too quickly for its kinetic behavior to be identified, and its concentration would apparently be strongly correlated with its instantaneous rate of production. This is an exaggeration, and none of the chemicals of interest exhibited this extreme behavior, but this is one end of the spectrum of behavior of the chemicals of interest, and consideration of such is important in fully understanding the analytical method.

At select times throughout the experiment, samples were taken from the test chamber reserved for supporting measurements including pigment concentrations and cell carbon and nitrogen content. Chlorophyll a and carotenoids were measured immediately following the first VOC time-point and every eight hours thereafter. At each time-point, 3, 4, and 5 mL of culture were vacuum filtered through glass fiber filters. The filtrate from the three samples was combined and re-filtered to account for pass-through (blank). These four filters were frozen and pigments were extracted with 90% acetone the next day according to the procedure outlined in Zavřel et al. (2015), and using the regression equation for cyanobacteria determined by Ritchie (2006). Spectrometry was done on a Shimadzu UV-2450 UV-Vis Spectrometer (Shimadzu Corp., Kyoto, Japan).

Cell carbon and nitrogen contents were measured three times throughout the day--once immediately after the first VOC time-point, again at eight hours after the start of the experiment, and again at sixteen hours after the start of the experiment. At each time-point, 3, 4, and 5 mL aliquots of culture were vacuum filtered through pre-combusted glass fiber filters. The filtrate from each of the three samples was combined and re-filtered to account for DOM (blank). The four filters from each time-point were frozen and processed in one batch at a later date via CHN analyzer (CE-440 Elemental Analyzer, Exeter Analytical, Inc., Chelmsford, MD, United States).

Results:

PTR-MS was used to measure the concentration of VOCs in the headspace of exponentially growing *Synechococcus* WH8102 cultures over a day-night cycle. VOC concentrations [in parts per billion by volume (ppbv)] are shown in Figures 1 through 4 and Tables 1 and 2. Figure 1 shows acetaldehyde concentrations measured in the f/2 +Si medium, the azide-killed cell

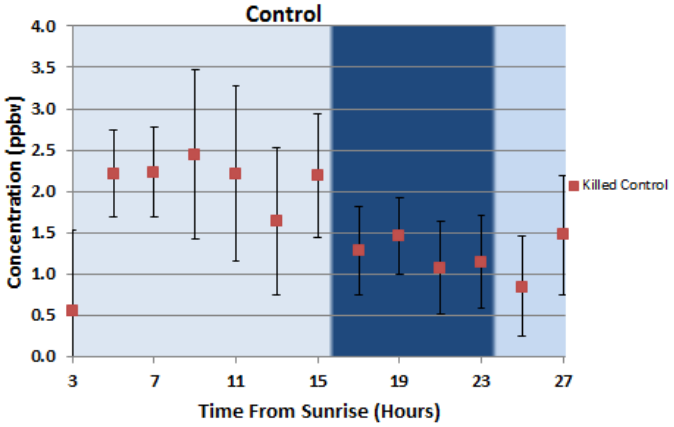


Acetaldehyde concentrations in air bubbled through blank F/2 + Si media (blank), a killed-cell control (killed), and live *Synechococcus* (live mean) (n=3). Error bars are 1 SD. Light and dark shading of the background indicate illumination of chambers.

control, and the average of the three live cell culture suspensions every two hours across the 24-h experiment. Acetaldehyde concentrations were among the highest measured. Other analytes present in high concentrations in the headspace gas included ethenone, ethanol, acetic acid, and dimethysulfide (DMS), as well as several VOCs corresponding to m/z values for which compound identities are not known (Tables 1 and 2).

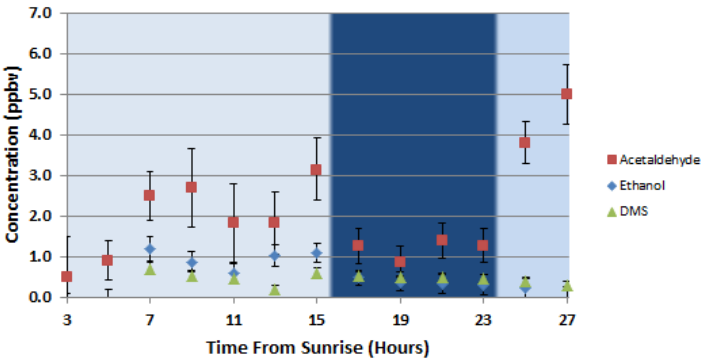
Each time-point carries two important numerical values: the difference between the signal from live culture and the signal from the killed control, and the difference between the killed control and the blank medium. In order for the data for a particular analyte to be informative, the concentration measured from the killed control must exceed that from the sterile f/2 + Si medium. This criterion removes contaminants or chemicals of abiotic origin from consideration. If the first criterion is met, the difference between the live culture and the killed control can be used to infer a deviation from first-order loss of analyte from the system. This information is used to assess the direct or indirect biological production or consumption of the analyte. When considering VOC production, not consumption, only cases where the live culture signal exceeded the killed control signal were deemed relevant. After confirming that the killed control exceeded the blank medium at all time-points, the data were displayed in a graph showing the live-culture signals minus the killed-control signals. For example, Figure 2 shows the killed culture minus the blank medium for acetaldehyde. Because every point is at or above zero, these acetaldehyde data are relevant at every time-point. All VOC graphs shown here, unless otherwise noted, had killed control signals

Figure 2: Acetaldehyde Blank-Subtracted Killed Control



Acetaldehyde concentrations in air bubbled through the killed-cell control (killed control) subtracted by the signal from the blank medium. Error bars are 1 SD. Light and dark shading of the background indicate illumination of chambers.

Figure 3: Live Minus Killed



Acetaldehyde, ethanol, and DMS concentrations in the live cultures minus the killed control. Error bars are 1 SD. Light and dark shading of the background indicate illumination of chambers. Points representing concentrations below zero were omitted. For acetaldehyde, groups of points which are not statistically different from one another (Welch's t-test, 99% confidence) are as follows: 7 and 9; 11 and 13; 17, 21, and 23. For ethanol, groups of points which are not statistically different from one another (Welch's t-test, 99% confidence) are as follows: 7, 13, and 15; 19, 21, 23, and 25. For DMS, groups of points which are not statistically different from one another (Welch's t-test, 99% confidence) are as follows: 9, 11, 17, 19, 21, 23; 23,25. All combinations of time-points not listed here are statistically different (99% confidence).

in excess of the blank medium signal at every point. Figure 3 shows the difference between live culture minus blank medium and killed culture minus blank medium signals for acetaldehyde, ethanol, and DMS.

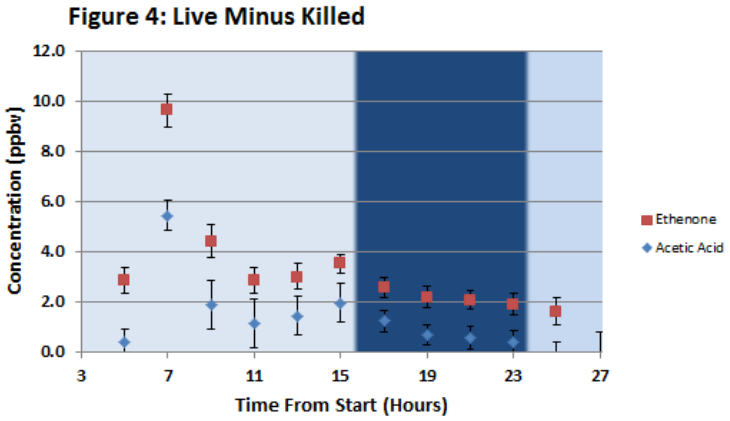
The data in figure 3 show changes in concentration throughout the 24-hour measurement period which, in many cases, exceed one standard deviation. All points above zero ppbv indicate production of the compound of interest by *Synechococcus* WH8102.

Acetaldehyde was only slightly above zero at hour three. It increased 5-fold over the first six hours of measurement, decreased slightly before increasing again just before the night period, stayed low and nearly constant in the night, and rapidly increased following sunrise.

The signal for ethanol started below zero. At hour seven it rose to about 1 ppbv, then stayed around 1 ppbv until hours thirteen and fifteen. During night, it exhibited a very slow decline from about 0.5 ppbv to about 0.3 ppbv, from which it did not recover in the first four hours of the next daytime.

The signal for DMS started below zero. At hour seven it rose to just below 1 ppbv, then declined almost to zero over the next six hours. At hour fifteen, DMS increased slightly and then plateaued through the night and into the first hours of the next day.

Figure 4 shows live-culture signals minus killed-culture signals for ethenone and acetic acid. Ethenone and acetic acid killed-control-adjusted signals followed a very similar pattern of concentration increases and decreases throughout the day. The signals both started below zero, passed zero by hour five, and reached their highest concentration by hour seven. Ethenone and acetic acid concentrations then declined for four hours, plateaued for four more hours, then slowly declined through the night and dropped below zero again in the first hours of the next day. The highest signal from ethenone occurred at hour seven, and was about 9.5 ppbv. The highest signal from acetic acid also occurred at hour seven, and was about 5.5 ppbv.

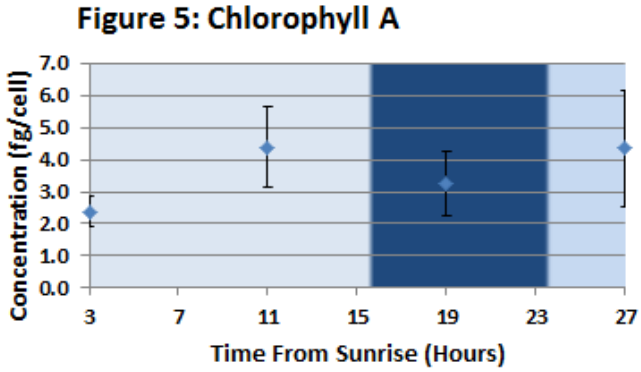


Ethenone and acetic acid concentrations in the live cultures minus the killed control. Error bars are 1 SD. Light and dark shading of the background indicate illumination of chambers. Points representing concentrations below zero were omitted.

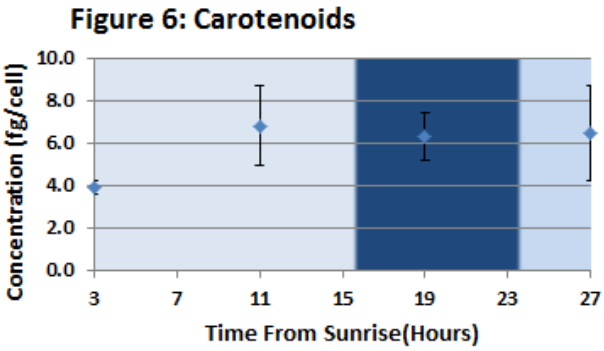
These few compounds show distinct fluctuations throughout the light/dark cycle. Several other m/z values gave interesting signals but compound identities are not known. These m/z values, 41, 101, and 164, correspond to exact masses of 40.038 ± .005 Da, 100.052 ± .005 Da, and 163.006 ± .005 Da. One possible chemical formula for m/z = 101 is $C_5H_8O_2$. Data for all compounds with minimal overlap between the standard deviations of the live-culture signal and

the killed-control signal are in Tables 1 and 2, displayed as live culture minus killed control in Table 1, and displayed as killed minus blank in Table 2.

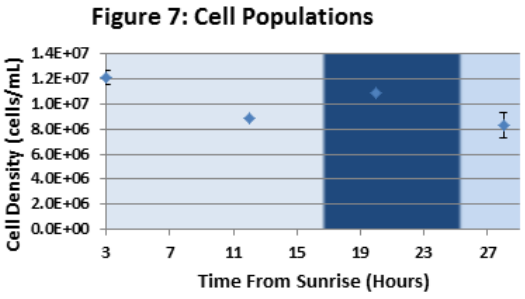
Supporting data were collected to determine the effects of bubbling on cell density, pigment concentrations, and carbon and nitrogen content. Chlorophyll a and carotenoid concentrations are shown in Figures 5 and 6. The concentrations remained relatively constant throughout the 24-hour period. If any change occurred, it was a slight increase in concentration to account for the change in daytime light levels before and after the start of the experiment. Cell densities in the chambers are shown in Figure 7. These values remained relatively constant, indicating little to no loss of cells due to bubbling. Carbon and nitrogen contents are shown in Figures 8 and 9. Carbon concentration is comparable to that found for *Synechococcus* WH8102 by Mouginot et al. (2015), but nitrogen is much higher here, likely due to the nutrient-replete medium.



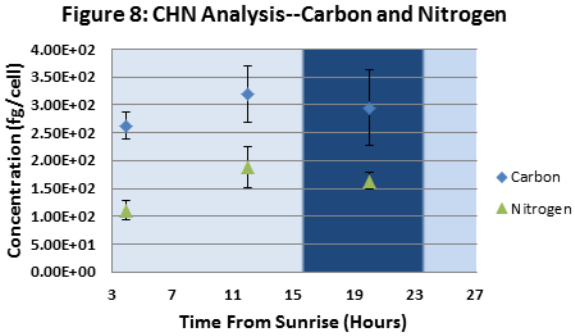
Concentration of chlorophyll A measured in the destructive-sampling live culture chamber throughout the day/night cycle. Error bars are 1 SD.



Concentration of carotenoids measured in the destructive-sampling live culture chamber throughout the day/night cycle. Error bars are 1 SD.



Cell density measured in the destructive-sampling live culture chamber throughout the day/night cycle. Error bars are 1 SD.



Concentrations of carbon and nitrogen measured in the destructive-sampling live culture chamber throughout the day/night cycle. Error bars are 1 SD.

Hrs from Dawn	3		5		7		9		11		13		15		17		19		21		23		25		27	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Unk 41	5.64	5.06	0.18	0.23	0.44	0.14	0.10	0.21	-0.12	0.22	-0.10	0.26	-0.18	0.19	-0.42	0.19	-0.24	0.18	-0.14	0.17	-0.23	0.19	-0.08	0.22	0.14	0.19
Ethenone	-16.94	6.10	2.86	0.50	9.63	0.66	4.41	0.66	2.86	0.53	3.00	0.51	3.52	0.37	2.57	0.38	2.19	0.43	2.07	0.39	1.90	0.42	1.63	0.54	-0.11	0.92
Acetaldehyde	0.52	0.98	0.92	0.48	2.51	0.60	2.71	0.97	1.83	0.97	1.83	0.76	3.14	0.76	1.28	0.43	0.89	0.39	1.39	0.44	1.28	0.43	3.80	0.52	4.99	0.72
Ethanol	-6.33	2.95	-0.05	0.26	1.21	0.31	0.87	0.25	0.61	0.23	1.03	0.27	1.09	0.24	0.49	0.18	0.36	0.19	0.34	0.23	0.31	0.25	0.24	0.25	-0.08	0.35
Isoprene	0.10	0.09	0.04	0.07	0.17	0.08	0.04	0.08	-0.05	0.12	-0.11	0.10	-0.25	0.12	-0.29	0.09	-0.29	0.10	-0.17	0.09	-0.32	0.09	-0.31	0.09	0.01	0.08
Acetic Acid	-18.33	5.12	0.41	0.36	5.45	0.78	1.88	0.52	1.15	0.48	1.46	0.42	1.95	0.44	1.24	0.32	0.68	0.36	0.58	0.32	0.44	0.32	-0.13	0.49	-2.46	0.80
DMS	-0.06	0.16	-0.20	0.15	0.72	0.16	0.54	0.14	0.46	0.13	0.22	0.10	0.61	0.11	0.52	0.10	0.52	0.11	0.50	0.10	0.47	0.11	0.40	0.10	0.31	0.10
Acetone	3.99	1.26	2.38	0.32	3.05	0.43	1.34	0.55	1.63	0.50	1.73	0.47	2.53	0.45	1.25	0.35	1.48	0.24	1.71	0.27	1.78	0.28	3.13	0.31	3.12	0.40
Phenol	-2.79	0.97	1.62	0.27	1.13	0.84	0.31	0.15	0.27	0.12	0.34	0.12	0.35	0.10	0.15	0.06	0.19	0.07	0.12	0.05	0.12	0.05	0.11	0.07	0.05	0.10
Unk 101	-0.17	0.06	-0.26	0.08	0.17	0.05	-0.14	0.17	-0.04	0.13	0.01	0.07	0.07	0.08	0.06	0.05	0.06	0.05	0.06	0.05	0.07	0.05	0.12	0.05	0.11	0.06
Unk 164	4.42	0.63	2.27	0.48	2.65	0.37	1.31	0.36	0.93	0.36	1.06	0.37	0.64	0.25	0.75	0.24	0.40	0.22	0.41	0.19	0.43	0.20	0.36	0.19	0.33	0.17

Table 1: Mean values and associated standard deviations for control-adjusted live signal (Live minus Killed) for each chemical of interest at each time-point. Units in parts per billion by volume of headspace air.

Hrs from Dawn	3		5		7		9		11		13		15		17		19		21		23		25		27	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Unk 41	1.0044	0.244	0.569	0.23	0.29	0.18	0.523	0.222	0.559	0.26	0.404	0.27	0.45	0.2	0.43	0.22	0.42	0.2	0.321	0.195	0.264	0.208	0.242	0.262	0.451	0.202
Ethenone	18.405	4.467	2.386	0.48	2.49	0.55	2.148	0.742	1.915	0.6	1.924	0.52	1.9	0.41	1.69	0.41	2.17	0.39	1.579	0.417	1.692	0.458	1.844	0.533	3.114	0.8
Acetaldehyde	0.5521	0.979	2.22	0.52	2.23	0.54	2.448	1.018	2.219	1.07	1.64	0.9	2.19	0.75	1.28	0.53	1.46	0.46	1.075	0.559	1.146	0.567	0.848	0.606	1.474	0.721
Ethanol	1.6783	3.003	0.896	0.31	0.72	0.3	0.562	0.317	0.382	0.26	0.705	0.31	0.57	0.26	0.53	0.25	0.65	0.21	0.474	0.269	0.47	0.295	0.561	0.286	0.768	0.344
Isoprene	0.1053	0.11	0.177	0.07	0.09	0.09	0.233	0.09	0.207	0.14	0.177	0.12	0.16	0.14	0.09	0.12	0.18	0.11	0.112	0.109	0.125	0.119	0.065	0.128	0.217	0.093
Acetic Acid	10.407	5.304	1.789	0.38	2.53	0.52	1.761	0.515	1.516	0.48	1.504	0.42	1.64	0.45	1.32	0.34	1.7	0.28	1.318	0.264	1.272	0.3	1.474	0.474	2.634	0.699
DMS	0.2833	0.198	0.594	0.17	0.17	0.15	0.23	0.155	0.166	0.15	0.15	0.12	0.2	0.13	0.15	0.11	0.16	0.13	0.148	0.121	0.174	0.119	0.151	0.115	0.118	0.118
Acetone	1.1925	0.521	2.236	0.34	0.77	0.37	3.27	0.591	2.295	0.62	1.927	0.53	2.24	0.44	1.74	0.41	1.55	0.29	1.342	0.318	1.391	0.312	1.107	0.343	1.572	0.414
Phenol	2.9737	0.973	0.136	0.07	0.08	0.06	0.134	0.07	0.12	0.07	0.143	0.06	0.11	0.05	0.12	0.06	0.12	0.07	0.098	0.063	0.112	0.057	0.104	0.072	0.197	0.101
Unk 101	0.1817	0.07	0.351	0.09	0.03	0.07	0.296	0.174	0.206	0.13	0.155	0.08	0.12	0.09	0.06	0.06	0.06	0.06	0.062	0.054	0.056	0.057	0.079	0.056	0.09	0.064
Unk 164	0.5621	0.341	1.028	0.28	0.21	0.1	0.846	0.227	0.848	0.22	0.782	0.2	0.61	0.15	0.54	0.15	0.52	0.16	0.403	0.149	0.432	0.146	0.421	0.148	0.353	0.131

Table 2: Mean values and associated standard deviations for blank-adjusted killed signal (Killed minus Blank) for each chemical of interest at each time-point. Units in parts per billion by volume of headspace air.

Discussion:

These data show that *Synechococcus* WH8102 produced an array of VOCs. For some of the chemicals, rates of production changed throughout the day, and appeared to be maximal during periods of active photosynthesis. This information backs up Halsey et al. (2017), and these results reveal a greater diversity of cellular VOC emissions than has been shown previously in marine algal cultures.

Not all compounds which were measured were included in the results. Many compounds were measured in nearly equal amounts in the live culture, the killed control, and the blank medium. VOC concentrations were reported if there were time-points for which the concentration in the live culture was more than one standard deviation greater than the concentration in the killed culture. Also, chemicals were excluded if their killed-control signals were less than their blank-medium signals.

Ethenone, also called ketene, and acetic acid show an interesting relationship. Ethenone reacts quickly with water to form acetic acid, and this reaction is reflected in the resemblance between the concerted fluctuations of these two chemicals' concentrations throughout the day (Hudson and Loeffler, 2013).

Ethanol was included in this study because of its metabolic proximity to acetaldehyde. Ethanol can be oxidized in one step, by alcohol dehydrogenases, to form acetaldehyde.

Dimethyl sulfide (DMS) is heretofore the most studied VOC in the biological/chemical oceanography community. It has been included in this study because it contains sulfur and because it has been studied widely before, but *Synechococcus* is not known to produce it (Malmstrom et al., 2005).

Phenol, or one of its isomers, was included in this study because it had a killed-control signal consistently higher than its blank-medium signal.

Because this study measured VOCs in axenic cultures in a laboratory setting, it measured VOC production as a result of the gene expression that is inherent to axenic *Synechococcus*. This research provides a useful starting point for understanding the VOCs produced by *Synechococcus* in a marine environment, but the picture is by no means complete. *Synechococcus* is known to alter its gene expression in co-cultures compared to monoculture (Omneya 2017). As such, the VOC production by *Synechococcus* in co-culture with other cells may be somewhat different from that measured here.

An important consideration to make when using this method is that VOCs may be produced by non-biological means in the bubbling chambers. Abiotic VOC production was accounted for by using a killed control. Abiotic production is best exemplified by DMS, which is barely produced by *Synechococcus* and is not known to be produced by strain WH8102 (Corn et al. 1996; Keller et al. 1989; Malmstrom et al., 2005). DMS can be produced by the photodegradation of dimethyl sulfoniopropionate (DMSP), which is present in phytoplankton (Slezak et al., 2007; Iyadomi et al. 2016; Turner et al. 1988). Even though DMS is not known to be produced by *Synechococcus* WH8102, DMSP is, and its photodegradation in the bubbling chambers may have produced the DMS signal seen here. Abiotic production of VOCs is accounted for by the control, but the indirect production of DMS occurring in this case is detected by PTR-MS in the same way as direct production. Arguably, however, whether a chemical is produced enzymatically or by abiotic reaction of biogenic does not matter; in order to be of ecological significance, all that matters is that production occurs.

Another potential shortcoming of this method is also indicated by the DMS data. Production only occurs when DMSP and light occur simultaneously, because the abiotic breakdown of DMSP into DMS is catalyzed by light. However, the nighttime (dark) datapoints for DMS are not much lower than the daytime (light) datapoints. This likely occurs due to a lag between production of DMS and its departure from the solution via bubbling. Different compounds have different affinities for the medium, the chamber walls, and the cells, and will therefore have different propensities to be retained in a bubbled solution. This phenomenon could potentially result in a disparity between production time and measurement time, but deviations are buffered by the two-hour delay between bubbling time-points.

Furthermore, fluctuations in measured concentrations are observed in the shifting baseline in the blank medium. The rate of loss of a given compound from the bubbled solutions should be described at first approximation by a first-order rate law. This is the case for each five-minute interval of bubbling. As the concentration of a given chemical is depleted, the rate of loss is reduced. Throughout the day, though, a first order rate law was not always observed for the killed control. Deviations from first-order behavior in the chambers which contain sterile medium indicate that the precision of these measurements is not perfect.

Despite these potential shortcomings, these results are useful to understand VOC dynamics in Despite these potential shortcomings, these results are useful to understand VOC dynamics in *Synechococcus* WH8102. Photodegradation is largely accounted for by the killed-cell control. This is indeed the case for DMS during the first few time-points; at times 3 and 5,

the live-cell DMS signal is less-than-or-equal-to the killed-control DMS signal, suggesting the absence of instantaneous production of DMS by the live cells. At later time-points, though, continued production of DMSP in the live culture causes the live-culture DMS signal to exceed the killed-control DMS signal. At these later time-points, the indirect nature of DMS production becomes irrelevant, and a difference between the live-culture signal and the killed-control signal develops. Importantly, the live-culture signal always exceeds the blank medium signal, suggesting that the live cells are indeed playing a role in the evolution of DMS in the bubbling chambers.

Other compounds also exhibited a higher signal in the live culture than in the other chambers. Ethenone, ethanol, and acetic acid both followed a similar trend to DMS, where the signal for the first few time-points was greater in the killed-control than in the live culture. This phenomenon does not mandate the existence of photodegradation, it only requires that there is a reservoir of these chemicals stored in the killed-control that exhausts only over time. As the killed-control signal for these compounds is reduced through the day, the live-culture values fail to follow suit, and production is indicated by the difference between the two values at a given time-point. Because both the killed-control and the live-culture signals almost always exceed the media blank for these chemicals, there should be little doubt that the increased concentrations of these chemicals in the medium is attributable to cellular action, either direct or indirect.

Synechococcus WH8102 produced an array of VOCs during a day/night cycle, with most of the VOCs targeted in this study being produced during periods of active photosynthesis. The growth conditions utilized here are comparable to conditions in the subtropical open ocean; the cells were nutrient replete, and reach a stationary phase after exhaustion of their nutrients in a manner comparable to the peak of a bloom. As such, it is likely that the chemicals of interest in this study will be produced by *Synechococcus* WH8102 in the ocean. Admittedly, there are some important differences between this microcosm experiment and the real-world conditions of an ocean bloom. Cells in an axenic batch culture are not exposed to predation, they do not interact with other phytoplankton, and they have relatively stable environmental conditions. Continued inquiry into the effects of interactions between multiple species of phytoplankton or between phytoplankton and heterotrophs would provide useful insight, but single-species isolates are a valuable starting point. Taking these data alone, some significant mechanisms for the escape of organic carbon from the biotic systems of the ocean into the atmosphere likely exist, and should be investigated.

References:

Corn M. et al. (1996) Origin and Importance of Picoplanktonic DMSP. In: Kiene R.P., Visscher P.T., Keller M.D., Kirst G.O. (eds) *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds*. Springer, Boston, MA

de Bruyn, W. J. et al. (2017) The biological degradation of acetaldehyde in coastal seawater. *Marine Chemistry*. 192: 13-21.

Halsey, K.H. et al. (2017) Biological cycling of volatile organic carbon by phytoplankton and bacterioplankton. *Limnology and Oceanography*. 62(6): 2650-2661.

Hudson, R.L. and Loeffler, M.J. (2013) Ketene formation in interstellar ices: a laboratory study. *The Astrophysical Journal*. 773(2).

Kanakidou, M. et al. (2005) Organic aerosol and global climate modelling: a review. *Atmospheric Chemistry and Physics*. 5: 1053-1123.

Keller, M.D. et al. (1989) Dimethyl Sulfide Production in Marine Phytoplankton. *Biogenic Sulfur in the Marine Environment.* ACS. Pp 167-182.

Malmstrom R. R. et al. (2005) Dimethylsulfoniopropionate (DMSP) assimilation by Synechococcus in the Gulf of Mexico and northwest Atlantic Ocean. *Limnology and Oceanography*. 50(6): 1924-1931.

Mouginot, C. et al. (2015) Resource allocation by the marine cyanobacterium SynechococcusWH8102 in response to different nutrient supply ratios. *Limnology and Oceanography*. 60(5): 1634-1641.

Omneya A. O. (2017) Interactions of Freshwater Cyanobacteria with Bacterial Antagonists. *Appl. Environ. Microbiol.* 83(7): e02634-16

Ritchie, R. (2006) Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynth. Res.* 89: 27-41

Schlundt, C. et al. (2017) Oxygenated volatile organic carbon in the western Pacific convective center: Ocean cycling, air-sea gas exchange and atmospheric transport. *Atmospheric Chemistry and Physics*. 17(17): 10837-10854.

Six, C. et al. (2004) Photophysiology of the marine cyanobacterium Synechococcus sp. WH8102, a new model organism. *Aquatic Microbial Ecology*. 35: 17-29

Slezak D. et al. (2007) Effects of solar radiation on the fate of dissolved DMSP and conversion to DMS in seawater. *Aquatic Sciences* 69(3): 377-393

Sun, J. et al. (2016) The abundant marine bacterium Pelagibacter simultaneously catabolizes dimethylsulfoniopropionate to the gases dimethyl sulfide and methanethiol. *Nature Microbiology*. 1:16065

Turner, S.M. et al. (1988) The seasonal variation of dimethyl sulfide and dimethylsulfoniopropionate concentrations in nearshore waters. *Limnology and Oceanography*. 33(3): 364-375.

Waterbury J. B. (1986) Biological and ecological characterization of the marine unicellular cyanobacterium Synechococcus. *Can. Bull. Fish. Aquat. Sci.* pp. 71-120

Zavřel, T., Sinetova, M. A. and Červený, J. (2015). Measurement of Chlorophyll a and Carotenoids Concentration in Cyanobacteria. *Bio-protocol* 5(9): e1467.

Zindler, C. (2013) Short-lived trace gases (DMS, isoprene, acetaldehyde and acetone) in the surface waters of the western Pacific and eastern Atlantic Oceans (Doctoral dissertation)