An examination of pelagic *Sargassum* community primary productivity and nutrient cycling in the face of Gulf Stream based energy

Lindsay L. Dubbs
UNC Coastal Studies Institute and
UNC Chapel Hill, Institute for the Environment
Wanchese, NC, USA
dubbs@email.unc.edu

Michael Piehler
UNC Coastal Studies Institute and
UNC Chapel Hill, Institute of Marine Sciences
Morehead City, NC, USA
mpiehler@email.unc.edu

Jonathan Colby
Verdant Power
New York, NY, USA
jcolby@verdantpower.com

***Abstract*— Pelagic *Sargassum* macroalgae entrained in the Gulf Stream serves as habitat for commercially and recreationally important fish and protected sea turtles, among other organisms. It also plays an important role in the sourcing of carbon, nitrogen, and phosphorus to the otherwise deficient oligotrophic pelagic waters of the Atlantic Ocean.**

***Sargassum* is seasonally concentrated along the western wall of the Gulf Stream off the coast of North Carolina. The Gulf Stream is a fast and constant western boundary current that consistently comes in close proximity to land off the coast of Cape Hatteras, North Carolina. This area is thus an area of interest for harnessing Gulf Stream energy.**

Gulf Stream turbines will likely be far removed from the surface of the water, and thus, the buoyant *Sargassum* that floats there. Nonetheless, the functional roles of Sargassum and its epibionts, including primary production and nutrient cycling, may be either negatively or positively influenced by Gulf Stream turbine wake.

Our research, conducted in mesocosms, examined how an increase in turbulence affected the primary production and nitrogen fixation by Sargassum macroalgae and its associated epibiotic communities.

Keywords—Gulf Stream, Sargassum, MHK energy, nitrogen fixation, primary productivity

I. INTRODUCTION

Introduction

Pelagic *Sargassum* macroalgae entrained in the Gulf Stream serves as habitat for numerous organisms, including commercially and recreationally important fish, and protected sea turtles [1]. It also plays an important role in the tranformation and fixation of carbon, nitrogen, and phosphorus to the otherwise deficient oligotrophic pelagic waters of the Atlantic Ocean. For instance, epiphytic cyanobacteria that utilize the extensive surface area of *Sargassum* fronds are responsible for fixing nitrogen [2, 3, 4] and bacterial metabolism remineralizes phosphorus [5], making these nutrients biologically available.

Sargassum is seasonally concentrated along the western wall of the Gulf Stream off the coast of North Carolina. The Gulf Stream current is a fast western boundary current, with peak velocities exceeding 2 m s^{-1}, and has the potential to provide substantial baseload power to the southeast coast of the continental United States [6, 7]. The Gulf Stream off the coast of North Carolina has been identified as an area with high potential for ocean current energy generation worldwide because it is a constant resource that shows the least variability in position in close proximity to land off the North Carolina coast as compared to other locations along the east coast of the United States north of the Florida Straits [8].

Sargassum floats at the surface of the water as a result of many pneumatocysts (air filled bladders) on healthy plants that make the algae positively buoyant [9]. Healthy *Sargassum* biomass is concentrated in the top 1 to 3 m of the water column (personal observation). Turbines designed to capture the available energy in the Gulf Stream, however, will likely be placed well below the surface of the water, at depths of 30 to 50 m, to avoid near-surface phenomena including cavitation, wave interactions, and commercial vessel traffic. Nonetheless, the functional roles of *Sargassum* and its epibionts may be influenced by the flow-structures and turbulence generated by turbine wake, which may affect nutrient cycling and productivity [10]. However, neither the magnitude nor directions of impacts are known. Even though *Sargassum* will be exposed to wake for short periods of time as it floats at the water's surface, entrained in the current, increased shear stress could cause cellular damage and

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removal of organisms from the surface of *Sargassum*, resulting in a potential negative impact of increased turbulence on these macroalgal communities. Another possible negative impact of exposing *Sargassum* to turbulence is a reduction in the nitrogen-fixing capacity of obligate anaerobic epibiont nitrogen fixing bacteria through oxygenation of the cells. There is also the potential that micro-scale changes in flow downstream of Gulf Stream turbines will increase productivity of the epibiont communities and *Sargasssum*. Turbulent flow increases mixing, thereby increasing nutrient supply to the water surrounding *Sargassum* fronds [11] and the uptake of those nutrients, as long as surrounding waters have higher nutrient concentrations than those in close proximity to *Sargassum* fronds.

Our research examined how an increase in turbulence intensity for 1 h affected the productivity of and nitrogen fixation by *Sargassum* macroalgae and its associated epibiotic communities in experiments conducted in two laboratory mesocosms. We expected that increased turbulence would increase productivity of *Sargassum* communities because of increased mixing, until nutrients were expended, whereupon productivity would decrease. In addition, we expected that increased turbulence would decrease rates of nitrogen fixation, because of increased intracellular oxygen levels, thereby exacerbating nutrient limitation of primary production.

II. Materials and Methods

A. Sample collection

Fishermen and fellow researchers working in the Gulf Stream provided information about presence/absence of *Sargassum* and prompted Gulf Stream trips when *Sargassum* was present. *Sargassum* and seawater were collected for laboratory experiments two times for this research. Sample collection trips on June 16, 2014 and May 5, 2015 were to approximately 35°32.2' N and 74° 44.6' W, along the western edge of the Gulf Stream off the North Carolina coast.

When *Sargassum* was encountered geographical location and physicochemical data was collected. A YSI (6600) was used to collect surface water temperature, which was used to determine the temperature to which *Sargassum* was exposed during laboratory experiments. Seawater was collected between 1100 and 1200 h in 5 gallon buckets and filtered through Nitex screen (53 micron) prior to storage in sealed 5-gallon buckets and transported approximately 4 hr to the laboratory at the UNC Coastal Studies Institute, Wanchese, NC. Subsamples of seawater were reserved for filtration through pre-combusted Whatman GF/F filters. 60 ml of filtrate were retained and stored on ice for subsequent nutrient analysis. Samples of pelagic Sargassum fluitans was collected using dip nets. Macrofauna were removed from the Sargassum fluitans prior to its storage in two insulated 5-gallon buckets filled with unfiltered seawater. A subsample of Sargassum was retained and stored in aluminum foil for subsequent dry weight analysis. Upon return to the laboratory, water and filter samples were frozen at approximately -12°C for future analysis. Sargassum samples were placed in a 60°C drying oven for at least 48 h prior to weighing.

B. Laboratory incubations

Prior to laboratory experiments, and within 12 h of sample collection, ~95 L (25 gal) of seawater was transferred to each of two mesocosms and *Sargassum* fronds were divided between the two mesocosms. Each mesocosm consisted of a ~102 L (27 gal) cylindrical polyethylene aquaculture tub (55.245 cm D x 45.72 cm H). Mesocosms were housed in a temperature-controlled environmental chamber outfitted with two 400-watt high-pressure sodium lamps connected to timers. The environmental chamber set-up allowed for replication of *in situ* water temperatures (24-25°C), light levels (approximately 1000 μmol s^{-1} m^{-2} of photosynthetically active radiation), and diurnal variations in light. The two mesocosms were set-up with the same axes for all measurements and identical flow conditions until increased turbulence was introduced in a single mesocosm for 1 h during each experiment. Flow was generated in each mesocosm using Power Stream Circulation Pumps (Sicce, Voyager 2, maximum flow rate of approximately 3 m^3 hr^{-1}). The circulation pump rotor in each mesocom was oriented parallel to the bottom of each mesocosm to promote mixing while minimizing the surface expression of the turbulent jet. However, during the 1-h turbulence addition, the angle of the circulation pump rotor in the turbulence treatment mesocosm increased by 10° relative to the bottom of the mesocosm to increase the turbulence intensity at the water's surface. The turbulence treatment mesocosm circulation pump was otherwise oriented identically to the control mesocosm circulation pump (Figure 1). The orientation of the circulation pumps was kept constant in the appropriate positions throughout and between experiments. Hereon, results will be referred to as "pre-turbulence" for samples collected prior to this 1 h increase in turbulence in the treatment mesocosm, and "post-turbulence" for samples collected after the 1 h increase in turbulence in the turbulence treatment mesocosm.

Laboratory experiments assessing rates of primary production and nitrogen fixation commenced within 24 h of sample collection. Rates of primary production by *Sargassum* and its associated epibionts and planktonic primary producers in the seawater, and nitrogen fixation of *Sargassum* and its associated epibionts were assessed pre-turbulence and post-turbulence. Rates of net primary production by *Sargassum* and its associated epibionts and planktonic primary producers were assessed by oxygen generation in glass bottles exposed to light [12] containing either *Sargassum* and plankton or plankton alone. Oxygen concentrations were determined optically (Hach HQ40d portable meter with LD0101 rugged optical dissolved oxygen probe). The light/dark bottle incubations were conducted in 300 ml BOD bottles (Wheaton) placed in water baths in the environmental chamber containing mesocosms for 2 h. Nitrogen fixation activity was tested using the acetylene reduction technique [13, 14] where the production of ethylene over time can be used as a proxy for rates of nitrogen fixation. Meristems of *Sargassum* and

approximately 405 ml of seawater or 405 ml seawater alone was added to 500 ml wide-mouth glass media bottles (Kimax). Bottles were capped with PTFE caps and bioassays were begun by replacing 10% of the bottle headspace with acetylene gas in *Sargassum* and seawater + acetylene and seawater + acetylene treatments. Another treatment consisting of seawater, *Sargassum* and no acetylene was also prepared to determine the evolution of ethylene in *Sargassum* without addition of acetylene. Acetylene reduction incubations were conducted in water baths located in the environmental chamber and bottles were gently mixed every ½ h for 4 h. Gas samples from immediately after commencement of the acetylene reduction bioassays and 4 h later were stored in evacuated serum bottles for future gas chromatographic ethylene analysis.

Subsamples of water and *Sargassum* were collected prior to each light/dark bottle and acetylene reduction experiment and from each treatment at the conclusion of each experiment. Water samples were filtered through pre-combusted Whatman GF/F filters and 60 ml of filtrate was retained and frozen at -12°C for subsequent dissolved ammonia, nitrate/nitrite, and orthophosphate concentrations. *Sargassum* subsamples were placed in aluminum foil and a 60°C drying oven for at least 48 h for subsequent dry weight analysis.

C. *Mean flow and turbulence measurements*

The mean flow field and turbulence intensity was assessed using a Nortek Vector Current Meter in the control mesocosm and in the turbulence treatment mesocosm set-up during the 1 h increased turbulence treatment. Ten-minute velocity measurements (x, y, and z directions; m s^{-1}) at a sampling rate of 32 Hz were made every 5 cm ($1/10^{th}$ of the diameter) from the middle of each water-filled mesocosm along each diameter transect (Fig. 1). Measurements were made at a depth of 5 cm below the surface of the water (Fig 1). The angle of the positive x_{ADV} axis relative to the positive x_m axis was 38.5°.

D. *Sample analysis*

Filtered (Whatman GF/F filters) water samples were analyzed for NO_3^--N, NH_3-N, and PO_4^{3-}-P by automated flow injection colorimetry (Lachat QC-8500 series 2 Automated Ion Analyzer) using the Cu–Cd reduction, phenol hypochlorite, and antimony-phosphomolybdate complexation methods, respectively (Parsons et al., 1984). Gas samples were analyzed for C_2H_4 by flame ionization detection gas chromatography (Shimadzu GC-2014). Sample separation was accomplished on a 2-m length, ~ 0.22 mm inner diameter Hayesep-Q column with an ultrahigh purity N2 carrier (25 mL min−1). Injector and detector temperatures were set at 100°C and 250°C. Biomass of *Sargassum* fronds was measured as dry mass.

E. *Calculations*

Average rates of net primary production of *Sargassum* were calculated as the average change in concentration of dissolved oxygen (mg/L) in light bottles containing *Sargassum* and seawater (n=2) minus the average change in concentration of dissolved oxygen (mg/L) in light bottles containing seawater alone (n=1 in June 2015, n=2 in May 2015) over time. These rates (mg O_2 L^{-1} h^{-1}) were normalized for the dry weight of Sargassum (g_{drywt}) in each sample bottle and were converted to rates of carbon fixation (mg C L^{-1} h^{-1} g_{drywt}^{-1}) by multiplying by stoichiometric factor of 1.1 [15].

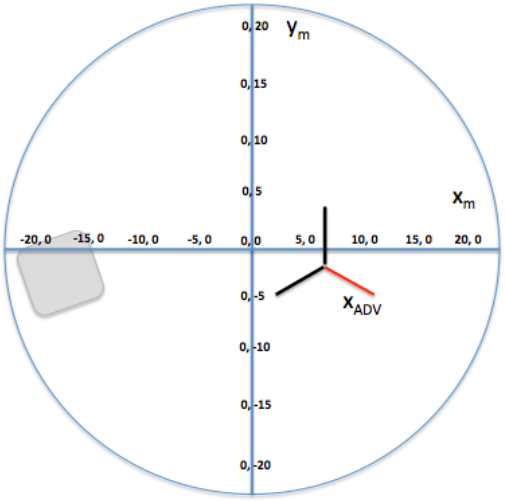

Fig. 1. Acoustic Doppler Velocimeter (ADV) sampling points and circulation pump (grey box) and ADV orientation in mesocosms used in a turbulence experiment where *Sargassum* from the Gulf Stream off the NC coast was subjected to increased turbulence in a laboratory experiment. Both the control and the increased turbulence mesocosms had the same axes and circulation pump location. The x-axis of the ADV (x_{ADV}) is red. Velocity measurements were made at 5 cm increments (x_m, y_m) from the center of each mesocosm across diameter transects (~55 cm).

Rates of acetylene (C_2H_2) reduction/ethylene (C_2H_4) evolution (µmol C_2H_4 L^{-1} h^{-1} g_{drywt}^{-1}) were calculated from the time linear changes of C_2H_4 concentrations in sample bottle headspaces over time. *Sargassum* + acetylene treatment (n=2) rates of were averaged for each treatment and experiment. Acetylene reduction served as a proxy for nitrogen fixation, which has been observed to occur at a stoichiometric ratio of C_2H_2:N_2 of between 3 and 4 (Hardy et al. 1973) for bacteria and algae. A stoichiometric ratio of 3.5 will be used for the purposes of this study.

The individual mean velocity components and standard deviations in each of the ADV x, y, and z directions at each 5 cm sampling point along the x and y mesocosm diameter axes (n=19200 samples for each direction) were calculated, and the turbulence intensity for each component at that point was calculated as the quotient of the standard deviation divided by the mean. Individual mean velocity components (V_x, V_y, and V_z) were then used to calculate the square root of the sum of squares, resulting in the total mean water velocity (V_W). The total turbulence intensity (%) at each point of measurement (x_m, y_m) was calculated as the quotient of the standard deviation of the total mean water velocity measurements divided by the total mean water velocity.

Treatment-wise and pre- and post-treatment differences between rates of primary production and nitrogen fixation were determined by confidence interval overlap (α=0.05).

III. Results and Discussion

A. Turbulence

The turbulence intensity measured in the mesocosms was measured 5 cm from the surface and *Sargassum* biomass in the mesocosms was concentrated within the surface 10 cm of each mesocosm. In the open ocean, *Sargassum* is concentrated near the surface within the top 1 m to 3 m of the water column. As such, the location of our turbulence intensity measurements in the mesocosm can be considered representative of measurements made within 1 m of the ocean surface. At this depth, turbulence intensity levels, and the associated turbulent mixing, are expected to be elevated [16].

Measured turbulence intensity in the control mesocosm ranged from 19% to 53%, with an average intensity across all points of measurement of 39%. Turbulence intensity in the turbulence treatment mesocosm during the 1 h of increased turbulence ranged from 38% to 69%, with an average intensity across all points of measurement of 51%. The average turbulence intensity in the control mesocosm was nearly equal to the minimum turbulence intensity in the increased turbulence mesocosm and was 75% lower than the average turbulence intensity in the increased turbulence mesocosm. The mean flow field in the control mesocosm was nearly 2 dimensional and constant in direction, circulating water in a counter-clockwise direction around the mesocosm (Fig. 2). However, the mean flow field in the increased turbulence mesocosm shows a significant change in the recirculation pattern with a significant amount of 3 dimensional upwelling due to the change to the circulation pump's angle (Fig. 2).

The turbulence intensities 5 cm below the water's surface in the increased turbulence mesocosm were higher at most locations measured, as compared to in the control mesocosm. Thus, *Sargassum* floating at the water's surface was subjected to more turbulence in the increased turbulence mesocosm, as was intended. The turbulence intensities reported here for both the control and turbulence treatment are higher than ambient turbulence intensity levels reported for tidal straits with similar mean velocities in the absence of a wave climate, TI ~ 15% - 20% at V_W ~ 2 m s^{-1} [17]. However, at present, little data exists in the literature to compare *in situ* turbulence intensity in the near-surface Gulf Stream current, with our without the installation of hypothetical Gulf Stream hydrokinetic turbines. As such, the level of turbulence simulated in the control mesocosm experiment can be considered as reasonable for the near-surface (1 m depth) of the open ocean. Further, the elevated levels of turbulence simulated in the turbulence treatment mesocosm are a conservative estimate of the open ocean turbulence intensity – perhaps representative of a significant storm event – and represent a very conservative estimate of elevated turbulence levels seen in the wake of a marine energy converter. We hope to be able to make these comparisons between our experimental conditions and real-world Gulf Stream turbulence intensities in the near future.

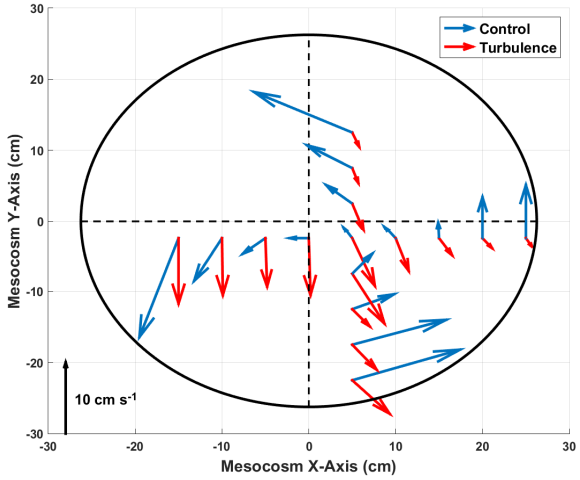

Fig. 2. Total mean water velocity (cm s^{-1}; 10 min average of 32 Hz data) at sampling points along the x and y axes of control (red) and increased turbulence (blue) mesocosms. Measurements were made 5 cm below the water's surface. Direction of arrows signifies the predominant direction of flow and the length of the arrows demonstrate the magnitude of velocity.

B. Patterns in Sargassum primary production

Average rates of net primary production by *Sargassum* and its epibionts ranged from 0 µg C g^{-1} h^{-1} $g_{dry\ mass}^{-1}$ (turbulence treatment post-turbulence, June 2014) to 4881 µg C g^{-1} h^{-1} $g_{dry\ mass}^{-1}$ (pre-turbulence, May 2015), with an average across both dates and all treatments of 1324 µg C g^{-1} h^{-1} $g_{dry\ mass}^{-1}$. Rates of primary production measured during this study span the range of measurements from a previous study ([18]; 181-1,234 µg C g^{-1} h^{-1} $g_{dry\ mass}^{-1}$) and encompass the rate measured by Howard and Menzies (1969; 700 µg C g^{-1} h^{-1} $g_{dry\ mass}^{-1}$). Net primary productivity by phytoplankton (light bottles not containing *Sargassum*; turbulence treatment, post-turbulence, May 2014) and the rate was negligible (48 µg C g^{-1} h^{-1} $g_{dry\ mass}^{-1}$).

The rates of net primary production by *Sargassum* were significantly lower after turbulence was introduced, in both the turbulence treatment and control in June 2014 (Fig. 3). In May 2015, the rates of net primary production by *Sargassum* were also lower after turbulence was introduced, but the difference was not significant for either the turbulence treatment or the control. These results suggest that nitrogen or phosphorus became limiting to primary production by *Sargassum* over time in the mesocosms, or that there was

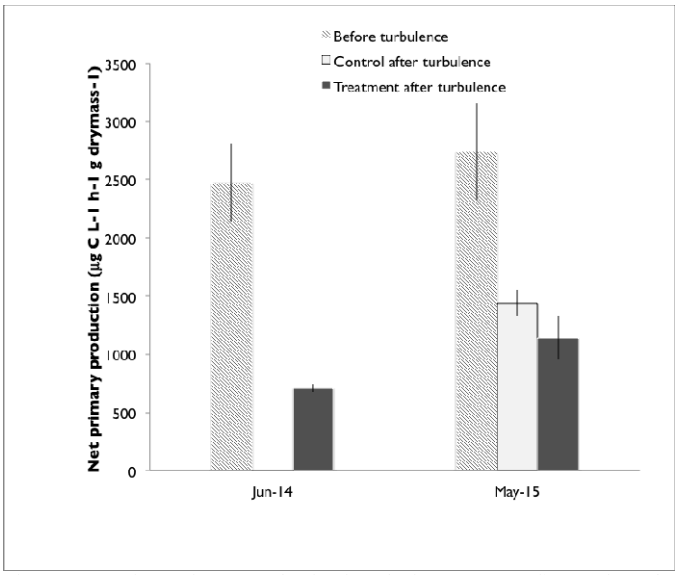

Fig. 3. Rates of net primary production in pelagic *Sargassum fluitans* from the Gulf Stream off the North Carolina coast before and after turbulence in control and increased turbulence treatment mesocosms during June 2014 and May 2015 experiments. Error bars represent 95% confidence intervals.

some other experimental relic that cause reduced rates of net primary production over time. Dissolved inorganic nitrogen

(DIN; ammonia + nitrate/nitrite) and soluble reactive phosphorus (SRP; orthophosphate) concentrations of seawater at the termination of primary productivity experiments did not support the case for dissolved inorganic nitrogen or phosphorus limitation (Table 1). DIN and SRP concentrations increased over time in both the June 2014 and May 2015, with the exception of the orthophosphate concentration in the turbulence treatment in May 2015, which increased and then leveled off from the beginning to end of the primary productivity incubation (Table 1). Future nutrient addition experiments will more clearly determine nutrient limitation of net primary production by *Sargassum* and its epibionts. We attempted to conduct experiments within a very short timeframe because mesocosm incubation experimental relics were expected to increase over time. Even so, there appear to be relics that present within less than 48 h of Gulf Stream *Sargassum* and seawater sample collection.

The rate of net primary production by *Sargassum* in the turbulence treatment post-turbulence was significantly higher than that by the control *Sargassum* post-turbulence in June 2014, but was slightly lower than the control rate in May 2015, although the difference was not significant (Fig. 3). These results suggest that we accept the null hypothesis that increased turbulence does not influence rates of primary production by *Sargassum fluitans* and its epibiont communities.

TABLE 1. Dissolved inorganic nitrogen (ammonia, nitrate, and nitrite) and soluble reactive phosphorus (orthophosphate) concentrations (µmol L^{-1}; n=2) pre- and post-turbulence in June 2014 and May 2015 experiments assessing rates of net primary production by *Sargassum* and its epibionts (S+L bottles) from the Gulf Stream off the North Carolina coast. Pre-turbulence nutrient concentrations are from approximately 24 h after samples were collected and post-turbulence nutrient concentrations are from experiments that terminated approximately 5-6 h later.

Experiment and treatment	**Average nutrient concentrations**		
	Ammonia ***(µmol L^{-1})***	Nitrate/nitrite ***(µmol L^{-1})***	***Orthophosphate/SRP (µmol L^{-1})***
June 2014 pre-turbulence	1.3±0.43	0.12±0.15	0.06±0.01
June 2014 control post-turbulence	1.0±0.56	1.8±0.55	0.09±0.02
June 2014 turbulence post-turbulence	0.81±0.24	1.3±0.13	0.05±0.00
May 2015 pre-turbulence	1.42±0.09	0.35±0.5	0.14±0.01
May 2015 control post-turbulence	3.7±0.21	0.84±0.24	0.31±0.03
May 2015 turbulence post-turbulence	2.7±0.92	0. 29±0.05	0.20±0.0

Nitrogen fixation showed strong dependency on the presence of *Sargassum* and light. Ethylene was not produced in the absence of *Sargassum* (Acetylene and seawater treatment) in any of the experiments, indicating that nitrogen fixation is associated with *Sargassum* and its epibionts, not free-living algae or bacteria in Gulf Stream seawater. Ethylene was also not produced in an experiment conducted in the dark in June 2014, whereas ethylene evolution was observed in *Sargassum* + acetylene treatments conducted under before and after the dark experiment. Production of ethylene by *Sargassum* in incubations where acetylene was not added did not exceed 8 nmol C_2H_2 L^{-1} h^{-1} g_{drywt}^{-1}, indicating that ethylene production by *Sargassum* not associated with nitrogen fixation is minimal and should not greatly influence calculations of nitrogen fixation rates.

Rates of ethylene evolution were significantly higher in May 2015 than June 2015 (Fig. 4), which suggests seasonality in the rates of nitrogen fixation in these pelagic communities. However, a more extensive study of pelagic and benthic *Sargassum* by Phlips et al. [13] indicated that month to month variability in rates of acetylene reduction were high, whereas there was not any seasonal pattern in rates of acetylene reduction by pelagic *Sargassum*.

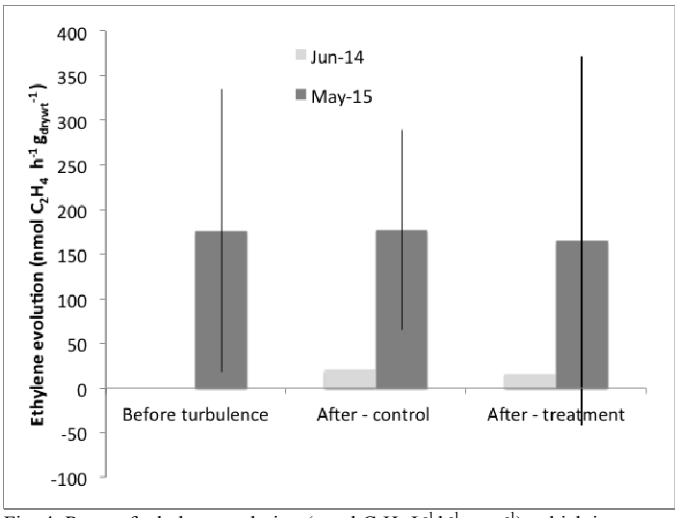

Fig. 4. Rates of ethylene evolution (nmol C_2H_4 L^{-1} h^{-1} g_{drywt}^{-1}), which is a proxy for nitrogen fixation, during acetylene reduction bioassays with *Sargassum fluitans* and its epibiont communities from the Gulf Stream off the North Carolina coast. Rates were determined pre- and post-turbulence in June 2014 and May 2015 mesocosm experiments. Error bars represent 95% confidence intervals.

June 2014 and May 2015 experiments were also different with regard to differences in the rates of nitrogen fixation from the pre-turbulence to post-turbulence experiments and between control and increased turbulence treatments post-turbulence. Rates of acetylene reduction by *Sargassum* communities significantly increased from the pre-turbulence to post-turbulence experiment in June 2014, and the rate of acetylene reduction following turbulence was significantly lower in the increased turbulence treatment than in the control. In May 2015, there were not significant differences between pre- and post-turbulence rates of nitrogen fixation by *Sargassum* communities nor were there significant differences in rates between treatments (Fig. 4). These results suggest that the impact of increased turbulence on nitrogen fixation by *Sargassum fluitans* communities is variable, although the nature of and reason for that variability is not known. Based on our results, the response may be the result of differences in *Sargassum* associated nitrogen fixing community activity or composition, which warrants further examination.

IV. Conclusions

Our results indicate that subjection of *Sargassum fluitans* and its associated epibiont communities to 1 h of turbulence at turbulence intensities averaging 51% has no significant impact on rates of net primary production or nitrogen fixation relative to *Sargassum fluitans* and its associated epibiont communities subjected to a constant average turbulence intensity of 39%. In laboratory experiments, we found that increased turbulence possibly introduced by the wake of Gulf Stream turbines did not decrease nitrogen or carbon fixation capacities of *Sargassum* communities.

Acknowledgments

Funding is provided by the North Carolina Renewable Ocean Energy Program. The authors would like to thank Corey Adams, John McCord, Molly Bost, Stephanie O'Daly, David Sybert, Jason Nunn, Farfrompuken Fishing charters, and the Albatross fleet for field and laboratory support. Drs. J.P Walsh and Reide Corbette graciously lent expertise and equipment for turbulence measurements. We are also grateful to Drs. Billy Edge and Nancy White, and the NC Renewable Ocean Energy Program Technical Advisory Committee for guidance and support.

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