Examining Metabolism in Seagrass Meadows: Trends in Respiration and Non-Dissolved Gas Ebullition

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Table of Contents

Abstract ……...…3
Acknowledgements ……..…5
Introduction ……6
Seagrass Ecosystems ……...6
Seagrass at the Virginia Coast Reserve on the Delmarva Peninsula ……..7
Oxygen Metabolism by Seagrass Ecosystems ……8
The Aquatic Eddy Covariance (AEC) Technique ……10
Thesis Outline ……...12
Literature Cited ……..…13
Chapter 1: Variation in seagrass meadow respiration measured by aquatic eddy covariance …..19
Abstract ……19
Introduction ……...19
Methods ……..…21
Results ……...24
Discussion ……26
Literature Cited ……29
Chapter 2: Non-dissolved Gas Fluxes in a Seagrass Meadow Measured using a Novel, Automated Bubble Trap...……..34
Abstract ……34
Introduction ……...34
Methods ……..…36
Results ……...44
Discussion ……54
Supplementary Materials ……..60
Literature Cited ……63
Conclusions ……...67
Literature Cited ……68
Appendix: VCR LTER Wind Study……..69

Abstract

Seagrass meadows provide many valuable ecosystem services, including carbon sequestration and storage of 'blue carbon'. Enhancing carbon cycling through seagrass restoration can thus act as a nature-based solution for climate change, as these ecosystems naturally remove carbon dioxide from the atmosphere through their high rates of productivity. Estimated metabolic values such as Gross Primary Productivity (GPP), Respiration (R), and Net Ecosystem Metabolism (NEM) are used in quantifying in situ carbon sequestration in seagrass meadows and other blue carbon ecosystems, as well as assessing ecosystem health. However, there are several assumptions within the methods and calculations that are conventionally used for estimating GPP, R, and NEM. In this thesis, I examine two of these assumptions: 1) respiration is constant and equal during day and night in calculations of GPP and R, and 2) dissolved oxygen (O_2) fluxes, which are used to estimate metabolic values, represent the total O_2 fluxes in the system and O_2 ebullition is negligible. These assumptions were tested in the restored eelgrass (*Zostera marina*) meadow at the Virginia Coast Reserve Long Term Ecological Research site (VCR LTER), located on the Eastern Shore of Virginia. To assess the first assumption, I examined a long-term data set of seagrass meadow O_2 fluxes collected using the aquatic eddy covariance technique, which allows for direct measurements of nighttime respiration. For the second assumption, I used a novel, automated bubble trap design to sample ebullition at a fine temporal scale and measured the content of O_2 and other gases in the collected gas volume to calculate O_2 flux in the form of bubbles.

In my first chapter, I found that respiration varied significantly over a 24-hour period in a seagrass meadow. Measurements showed a linear decrease in respiration of 29% through the night, from dusk to dawn. A corresponding linear increase in daytime respiration coupled with production described by a standard photosynthesis–irradiance curve accurately predicted measured daytime O_2 fluxes. The results of this study show that the assumption in metabolic estimates that night- and daytime respiration are constant and equal is not upheld. However, if respiration can be approximated as we found here by linear relationships, standard means for calculating daily metabolic numbers remain valid if estimates are based on full 24-hour records of O_2 flux data.

In my second chapter, I found that the O_2 bubble flux makes up a small percentage of total O_2 fluxes, only increasing daily GPP estimates by 2.4% in July, 3.6% in August. However,

even though accounting for O_2 ebullition would only increase total O_2 flux estimates by a few percent, it may still provide some value to incorporate into estimates of metabolic numbers. Some temperate seagrass meadows, like the one studied here, have been shown to be on the threshold between being net autotrophic and net heterotrophic in the past. The results also showed the variability in ebullition of the greenhouse gases CO_2 and CH_4, with this study being the first to our knowledge to quantify CO_2 ebullition in a seagrass meadow over a relatively long sampling period. More study is needed to understand the highly variable nature of CH_4 ebullition, a very strong greenhouse gas. Using the novel, automated bubble trap technique, it was possible to examine bubble fluxes at a fine temporal scale (every 10 minutes), which allowed for comparison with quickly changing environmental variables. We found that high light availability, supersaturated O_2 conditions, and low tides are the important drivers in stimulating non-dissolved gas flux.

In summary, I found that the assumption that respiration stays constant and equal between night and day was not supported, and that the assumption that non-dissolved O_2 fluxes are not a significant component of total O_2 fluxes is upheld. Improving the understanding of metabolism in a temperate seagrass meadow provides more insight into the role these ecosystems play in climate change mitigation through their in-situ carbon sequestration and storage potential.

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Introduction

Seagrass Ecosystems

Seagrass meadows are highly productive ecosystems that offer many benefits for marine life and coastal communities (Larkum et al., 2006; McGlathery et al., 2007). They provide essential ecosystem services, such as carbon sequestration and storage, nutrient cycling, coastal protection, enhancing biodiversity, and creating nurseries for commercially significant fish and shellfish (Cullen-Unsworth & Unsworth, 2013; McGlathery et al., 2007; Orth et al., 2006b; Reynolds et al., 2016). Seagrasses act as a foundation species, supporting thousands of marine organisms in various life stages (Beck et al., 2001). They also reduce coastal erosion that can be caused by storms; their rhizome structures help stabilize the sediment, and the leaves of seagrasses can form dense meadows that attenuate flow (Fonseca and Fisher 1986). Seagrass meadows are also exceptional at sequestering and storing carbon, which is important in the context of climate change mitigation.

The term 'Blue carbon' describes carbon that is sequestered and stored in vegetated coastal ecosystems, including in seagrass meadows (Nelleman et al., 2009). Seagrasses account for a significant portion of all marine carbon burial (10-18%), despite only covering a small area (0.1%) of the ocean floor (Greiner et al., 2013). Globally, seagrass ecosystems potentially store between 4,200 and 8,400 Tg of organic carbon in their sediments and 151 Tg of organic carbon in above and below-ground biomass (Fourqurean et al., 2012). They accumulate carbon in two ways. First, the meadow canopy reduces the flow velocity, allowing sediment particles that are often rich in organic matter to settle. These particles are less likely to get resuspended in the water column when they are in a seagrass meadow compared to bare sediment (Adams et al., 2016). Second, seagrasses also accumulate carbon through in-situ production (Greiner et al., 2013). This ability of seagrasses and other blue carbon ecosystems to sequester and store carbon is potentially important as a nature-based solution to reduce global atmospheric carbon dioxide concentrations (Duarte et al., 2013).

Large-scale and rapid losses of seagrass ecosystems are happening along coasts around the world. Seagrasses have been disappearing at a rate of 110 $km^2\ yr^{-1}$ since 1980, a loss of 7% yr^{-1} of total global seagrass extent (Waycott et al., 2009). These losses are due to sea level rise, sedimentation changes, water quality changes, eutrophication, extreme climate events, habitat fragmentation or destruction, invasive species, and other anthropogenic drivers (Orth et al.,

2006b). When seagrass ecosystems are lost, the carbon stored in their sediments will eventually re-enter the atmosphere and reverse the positive effect of seagrass carbon sequestration and storage (Salinas et al., 2020). Current rates of seagrass losses could lead to the release of 299 Tg of organic carbon yearly (Fourqurean et al., 2012). This makes seagrass meadows key targets for both conservation and restoration projects.

Seagrass at the Virginia Coast Reserve on the Delmarva Peninsula

The Virginia Coastal Reserve Long-Term Ecological Research site (VCR LTER), which is part of the Long-Term Ecological Research (LTER) network, is home to the largest and most successful seagrass restoration in the world. This restoration effort, which has been ongoing for two decades, covers over 17 km^2 of the shallow coastal bays of the VCR LTER (Greiner et al., 2013; McGlathery et al., 2012; Orth et al., 2006a). The VCR LTER is located on the Eastern Shore of Virginia, spanning 110 km along the Atlantic coast of the Delmarva peninsula, and covering tidal marshes, lagoons, and barrier islands.

The seagrass meadows in the shallow lagoons of the VCR LTER are predominately made up of a monoculture of one seagrass species: *Zostera marina*, or eelgrass. *Z. marina* is the most common species of seagrass in northern temperate areas, ranging from the Arctic to the Mediterranean and from North Carolina to the Gulf of California (Staehr & Borum, 2011). At its northern most limits, *Z. marina* is limited by low light conditions and hindered seed production, while at the southern most parts of its range, high temperatures inhibit productivity. The threshold for temperature stress for eelgrass has been found to be around 28°C under laboratory conditions (Staehr & Borum, 2011), and around 28.6°C under in situ field conditions (Berger, 2021). The Delmarva peninsula is near the southern-most limit of the distribution of *Z. marina*, and the bays of the VCR LTER frequently experience temperatures above the 28°C threshold during the summer months (Berger et al., 2020). In this part of its range, *Z. marina* also has a seasonal growth cycle. In the early spring through the middle of summer *Z. marina* grows rapidly. It begins to die back in the late summer, undergoing senescence through the fall (Clausen et al., 2014; Duarte, 1989).

Z. marina has been historically abundant in the bays of the VCR LTER, but seagrass wasting disease (*Labyrinthula zosterae*) as well as intensive storm activity in the early 20^{th} century caused massive declines in seagrass abundance (Orth et al., 2006a). In the northern bays

seagrass returned naturally over time, but the southern bays did not recover. In the 1990s a restoration project was started with the goal of transplanting seagrasses into the southern bays, followed by a seeding effort started in 2001 (Orth et al., 2006a). Now, the meadows in the southern bays have recovered (Figure 1) and reintroduced many of the important ecosystem services (Greiner et al., 2013; Orth et al., 2006a). The largest restored meadow is in South Bay, where our study site is located. South Bay is a shallow subtidal lagoon that is connected to the Atlantic Ocean by two inlets and is constrained by barrier islands to the east and the Delmarva peninsula to the west (Berger et al., 2020).

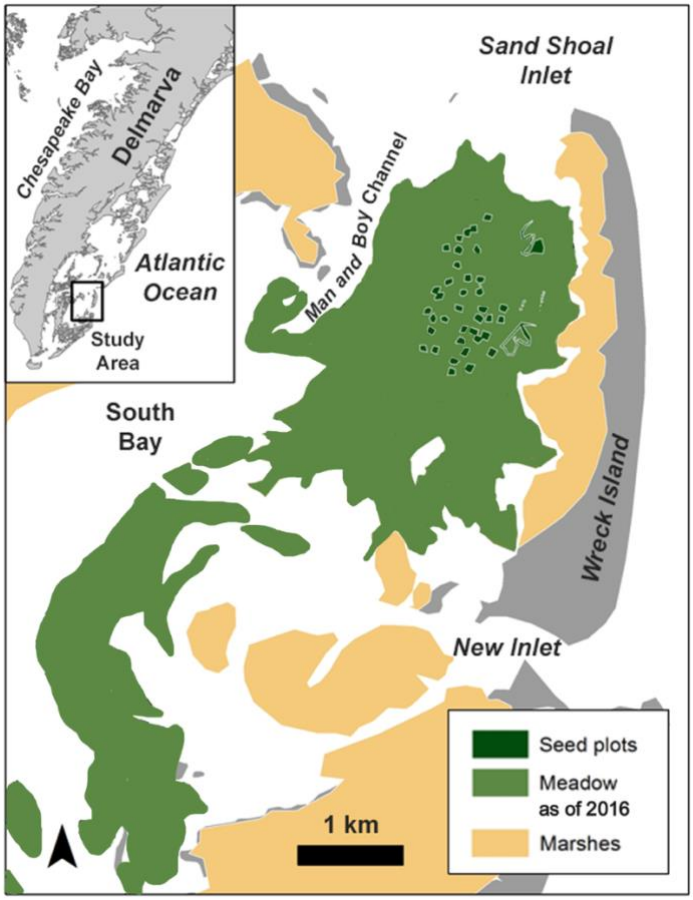

Figure 1. The South Bay seagrass meadow is located off the east coast of the Delmarva peninsula and is constrained by coastal marsh to the west and barrier islands to the east. This map shows the original seeding plots for the meadow restoration, as well as the extent of the meadow in South Bay as of 2016 (modified from Oreska et al. 2020).

Oxygen Metabolism by Seagrass Ecosystems

Seagrass meadows are predominately autotrophic ecosystems. On average across seagrass species, Gross Primary Production (GPP) tends to be higher than Respiration (R), with

mean (mean ± SE) values of GPP = 225 ± 11.1 mmol O_2 m^{-2} d^{-1} (n = 354) and R = 188 ± 10.1 mmol O_2 m^{-2} d^{-1} (n = 326) (Duarte et al., 2010). However, in the *Z. marina* meadows of South Bay in the VCR LTER, the balance between GPP and R has been shown to be close to the threshold between net heterotrophy and net autotrophy (Berger et al., 2020). Estimates of metabolic numbers, including GPP, R, and net ecosystem metabolism (NEM) in aquatic environments are usually found by measuring dissolved O_2 fluxes (Berg et al., 2019; Champenois & Borges, 2012; Koopmans et al., 2020; Mantikci et al., 2020; Ziegler & Benner, 1998). O_2 fluxes are used as a proxy for CO_2 exchange (Duarte et al., 2010), and can give us insight into ecosystem health and in-situ carbon sequestration.

Light availability drives O_2 fluxes in seagrass ecosystems (Duarte et al. 1991). Production increases linearly with increased light at low light levels but can curve and reach a maximum value at higher light levels, where light is no longer limiting. This light and production relationship for seagrass has been shown using a P-I (Photosynthesis – Irradiance) curve (Rheuban et al., 2014b). Other environmental drivers also impact O_2 fluxes. An increased current velocity can stimulate metabolic activity by reducing the diffusive boundary thickness along the seagrass leaves and enhances gas exchange (Fonseca & Kenworthy, 1987). Seagrass metabolism is also greatly affected by temperature stress (Berger, 2021; Rasmusson et al., 2020; Staehr & Borum, 2011). At a certain threshold, photosynthesis is reduced due to temporary damage of the photosynthetic components in the cell (Rasmusson et al., 2020).

Several studies have found that O_2 fluxes are greater in the morning at the same light levels than they are in the afternoon (Attard & Glud, 2020; Berg et al., 2019; Hanelt, 1992; Koopmans et al., 2020). To estimate metabolic numbers, including GPP, R, and NEM, several assumptions are made in the methodology and calculation process. These assumptions could explain the differences in O_2 flux in the morning and afternoon at the same light availability. One of these assumptions is that respiration stays constant and is equal between night and day. However, studies have speculated that respiration does vary over a 24-hour period (Howarth et al., 2014; Pace and Prairie, 2005; Pringault et al., 2009). Seagrasses produce highly labile compounds in the form of dissolved organic carbon exudates (DOCs), which have been shown in laboratory experiments to stimulate respiration (Moriarty & Iverson, 1986) and microbial activity (Gribsholt & Kristensen, 2002). Field studies in seagrass ecosystems have proposed that these compounds could be responsible for variable rates of respiration (Attard & Glud, 2020; Howarth

et al., 2014). It is likely that the consumption of these labile compounds could cause significant variation in seagrass respiration over the course of a day and could explain the observed hysteresis.

Another assumption that is made by common estimates of metabolic numbers is that total O_2 fluxes are well-represented by dissolved O_2 fluxes, and O_2 ebullition is negligible. Studies have suggested that non-dissolved sources of O_2 could be a significant part of the total system O_2 fluxes (Borum et al., 2007; Long et al., 2019). Fluxes of O_2 bubbles have been shown to be especially high in the afternoon in seagrass meadows (Long et al., 2019). If these non-dissolved O_2 fluxes are higher during certain times of day and are not included in total O_2 fluxes, this could potentially explain some of the observed differences in morning and afternoon fluxes under the same light conditions in seagrass ecosystems.

The Aquatic Eddy Covariance (AEC) Technique

The eddy covariance method, typically used for measuring atmospheric fluxes, has been adapted for use underwater (Berg et al., 2003) and has since been used in a variety of aquatic environments, such as permeable sediments (Berg et al., 2013; Huettel et al., 2020), rivers (Berg & Pace, 2017), deep ocean sediments (Berg et al., 2009), Arctic ice (Long et al., 2012), coral reefs (Long et al., 2013), oyster reefs (Volaric et al., 2018), algal beds (Attard et al., 2015), and seagrass meadows (Berger et al., 2020; Camillini et al., 2021). The aquatic eddy covariance technique (Figure 2) is noninvasive and can take O_2 flux measurements at spatial scales as large as 100 m^2 (Berg et al., 2007), estimating metabolism across a large area of the benthic surface. Because of this, the aquatic eddy covariance technique takes measurements under in-situ environmental conditions and is representative of heterogenous environments, such as a patchy seagrass meadow. Conventional methods of estimating seagrass metabolism, such as benthic in situ chambers exclude natural flow and do not necessarily replicate in situ light (Pedersen et al., 2013; Olivé at al., 2016). Many of these methods also scale-up measurements that are made over a small area and for short periods of time (Long et al., 2015; Martin et al., 2005). This leads to additional inaccuracies, as seagrass ecosystems have highly dynamic metabolic rates that can vary spatially and temporally. On the downside, the aquatic eddy covariance technique is more complex to use and also more expensive to adopt than conventional methods.

Figure 2. The aquatic eddy covariance technique deployed over a temperate seagrass meadow at the Virginia Coast Reserve LTER site (photo courtesy of Berg Lab).

With aquatic eddy covariance, we have been able to collect extensive data on seagrass ecosystems and estimate metabolic values such as GPP, R, and NEM (Rheuban et al., 2014a; Rheuban et al., 2014b), as well as understand the role environmental variables, such as temperature, play in seagrass productivity and health (Berger et al., 2020; Berger, 2021). However, calculating O_2 fluxes and metabolic numbers involves several assumptions. Hysteresis in dissolved O_2 fluxes, for example, indicates that there may be a need to evaluate the assumptions made with the commonly used methods and calculations. By examining these assumptions, we can create greater confidence in our techniques and develop an enhanced understanding of the dynamic metabolism in seagrass meadows.

Thesis Outline

Metabolic values that are important for quantifying system in-situ carbon sequestration and ecosystem health are estimated using O_2 fluxes. By using the aquatic eddy covariance technique, we have been able to create extensive datasets of O_2 fluxes and quantify GPP, R, and NEM in the temperate seagrass meadows of Virginia's coastal bays. In my M.S. thesis, I assess two of the assumptions made by the methods and calculations we use for quantifying metabolism in seagrass meadows and other aquatic ecosystems.

In my first chapter, I examine the variability of 24-hour respiration in an eelgrass (*Zostera marina*) meadow using long-term measurements of O_2 fluxes made with the aquatic eddy covariance technique, and make recommendations for accurate metabolic number estimates in aquatic systems based on our findings.

In my second chapter, I quantify non-dissolved gas (bubble) fluxes in a *Z. marina* meadow, assess the environmental drivers of these fluxes, and examine the composition of the collected gases, with special interest in O_2 ebullition. Additionally, I introduce a novel bubble trap design that allows for gas ebullition measurements at a fine temporal scale.

My first chapter has been submitted and is under initial review in the journal *Limnology and Oceanography Letters*. The second chapter will be submitted to *Limnology and Oceanography* or *Limnology and Oceanography Methods*. Exploring the assumptions used in estimating metabolic numbers will lead to a more complete understanding of these dynamic coastal ecosystems and could provide explanations for the observed hysteresis both in Virginia's coastal bays and other seagrass meadows around the world.

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Chapter 1: Variation in seagrass meadow respiration measured by aquatic eddy covariance

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Abstract

Accurate daily metabolic estimates of respiration, gross primary production, and net ecosystem metabolism are necessary to assess ecosystem health and blue carbon contributions of vegetated coastal ecosystems. Using our database of 2,115 hourly benthic oxygen (O_2) fluxes measured by aquatic eddy covariance, we examine how respiration for a *Zostera marina* seagrass meadow varies through night and day, and how this affects commonly performed metabolic estimates. Respiration decreased linearly by 29% through the night and a corresponding linear increase in daytime respiration coupled with production described by a standard photosynthesis–irradiance curve accurately predicted measured daytime O_2 fluxes. Many studies have questioned the widely used assumption in metabolic estimates that night- and daytime respiration are constant and equal. However, if respiration can be approximated as we found here by linear relationships, standard means for calculating daily metabolic numbers remain valid if estimates are based on full 24-hour records of flux data.

Introduction

Seagrass meadows are highly productive coastal ecosystems that provide many important ecological services, including sequestration and storage of carbon (Hemminga and Duarte, 2000). Seagrasses accumulate organic carbon in their sediments through in situ production as well as by accretion of carbon particulates from the water column. Thus, they act as a significant sink of and provide long-term storage for "blue carbon," carbon captured by coastal ecosystems (Nellemann et al., 2009; Mcleod et al., 2011; Greiner et al., 2013). This important ecological service of seagrass meadows has led to substantial restoration and conservation efforts as strategies for climate change mitigation.

To better inform mitigation strategies through carbon accumulation and storage, it is important to understand the dynamic metabolism of seagrass meadows (Berger et al., 2020). To do this, we need accurate and precise assessments of daily values for gross primary production

(GPP), respiration (R), and net ecosystem metabolism (NEM), which can be estimated from oxygen (O_2) fluxes. O_2 fluxes are a good proxy for the photosynthetic fixation of CO_2 because of the close association between carbon transformation and coupled O_2 production, and between carbon oxidation and O_2 consumption. The ratio between O_2 exchange and CO_2 exchange has been estimated to be approximately 1 (Kirk, 1983; Duarte et al., 2010), and is widely used in metabolic studies (Glud, 2008; Rheuban et al., 2014b; Berger et al., 2020).

Daytime O_2 fluxes for seagrass meadows can show deviations from what would be expected based on the amount of available light, which is the main control on O_2 fluxes during the day (Ralph et al., 2007). Several studies have found that O_2 fluxes in seagrass meadows show a higher net release of O_2 in the morning at the same light levels than in the afternoon (Hanelt, 1992; Rheuban et al., 2014b; Berg et al., 2017; Berg et al., 2019; Koopmans et al., 2020). Multiple explanations for this have been proposed, including depletion in water column CO_2 in the afternoon to a point where it limits photosynthetic production (Berg et al. 2019), increased afternoon water temperature (Masini and Manning, 1997; Rheuban et al., 2014a), photoinhibition (Hanelt, 1992), and enhanced consumption of highly labile organic compounds produced during photosynthesis that would cause respiration to vary over the course of a day (Glud, 2008; Howarth et al., 2014; Rheuban et al., 2014b; Attard & Glud, 2020)

In part because standard O_2 flux methods do not allow direct measurements of respiration during daytime, it is commonly assumed that respiration stays constant throughout the day and night in calculations of daily GPP and R (Hume et al., 2011; Rheuban et al., 2014a). Several studies have suggested this assumption may result in incorrect calculations of metabolic numbers (Pace and Prairie, 2005; Pringault et al., 2009; Howarth et al., 2014).

Respiration trends have been examined in other systems, with indirect approaches such as inferring respiration from water column O_2 concentration measurements combined with a full water column mass balance (Sadro et al., 2014; Mantikci et al., 2020), or isotope ratio measurements through time (Bender & Grande, 1987; Tobias et al., 2007; Venkiteswaran et al., 2008). However, these indirect approaches are subject to uncertainties, especially in a dynamic system, caused by air-water gas exchange and vertical and horizontal mixing. In this study we used a more direct approach, the noninvasive aquatic eddy covariance technique (Berg et al., 2003), to examine trends in respiration. Previous aquatic eddy covariance measurements have found respiration to decrease throughout the night in coral reef ecosystems (Long et al., 2013).

Here, we use our extensive database of 2,115 hourly benthic O_2 fluxes measured in a dense seagrass meadow at a single site within the Virginia Coast Reserve Long Term Ecological Research site (VCR LTER). This site has been subject to a series of metabolic seagrass studies (Hume et al., 2011; Rheuban et al., 2014a; Rheuban et al., 2014b; Berg et al., 2019; Berger et al., 2020). To our knowledge, this is the first study of variation in respiration in a coastal ecosystem that uses a long-term dataset of metabolic measurements performed under naturally varying environmental conditions.

Methods

The data used in this study spans 5 years (2014-2019) and compiles 161 days of seagrass (*Zostera marina*) metabolism measurements collected at the VCR LTER (Berg et al., 2019; Berger et al., 2020). The VCR LTER is the site of the largest and, to date, most successful seagrass restoration effort in the world. The most expansive meadow is located in South Bay (Orth & McGlathery, 2012) where the data was collected. This meadow encompasses 20 km^2 as of 2018 (Orth et al., 2020). South Bay is a shallow subtidal lagoon that is connected to the Atlantic Ocean by two inlets and is constrained by barrier islands to the east and the Delmarva peninsula to the west (Figure 1). Our site has a mean water depth of 1.2 m, with a tidal range of approximately 1 m. Seagrass at this site is perennial and seagrass shoot density varies seasonally. Z. marina grows rapidly from the early spring and through the middle of summer, and undergoes senescence starting in late summer and continuing through the fall (Clausen et al., 2014; Duarte, 1989). Mean June density at the center of the meadow for the study period was 416 ± 11 (mean ± SE) shoots m-2. In South Bay in June, mean aboveground biomass has been reported to be 50.7 g m-2 and mean belowground biomass has been reported to be 55.1 g m-2 (Berg et al., 2019).

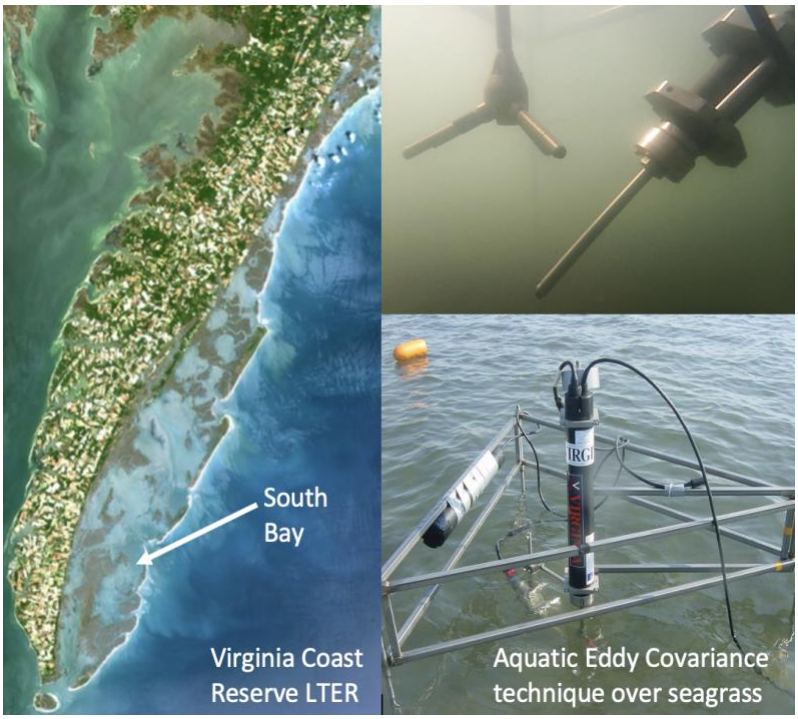

Figure 1. The data used here were collected at the Virginia Coast Reserve Long Term Ecological Research site in the South Bay eelgrass (*Zostera marina*) meadow, using the noninvasive aquatic eddy covariance technique.

The measured variables included in this study are O_2 flux, PAR (Photosynthetically Active Radiation) at the top of the seagrass canopy, current velocity, and water column O_2 concentration and temperature. The O_2 flux was measured above the seagrass canopy (at 30 cm above the sediment) using the aquatic eddy covariance technique (Berg et al., 2003), a noninvasive approach for direct O_2 flux measurements under naturally varying in situ conditions. The technique gives fluxes at a high temporal resolution (down to ~15 min) for a relatively large area of the benthic surface (typically 10 to 100 m^2) (Berg et al., 2007), and thus, integrates well over typical meadow patchiness (Rheuban & Berg, 2013). Older techniques for O_2 flux measurements, such as benthic in situ chambers, are not able to fully represent the natural system (by altering water movement and light diffusion) which can significantly affect the accuracy of metabolic estimates (Martin et al., 2005; Long et al., 2015; Olivé et al., 2016).

In our analysis, we included measurements from late March through late October. Data outside this period was excluded due to the large differences in the periods with daylight. The data were binned into mean hourly values for a full 24-hour period, and analyses were performed on the binned data rather than individual days. Hours were considered to be nighttime hours if

the PAR values were less than 1% of the maximum PAR during the day, a threshold that has been used in previous metabolic seagrass studies (Hume et al., 2011). This definition gave a well-defined separation between day and night at 20:00 in the evening and 06:00 in the morning.

Temperature stress has been shown to occur in *Z. marina* at temperatures above 28°C, where photosynthetic processes are inhibited (Staehr & Borum, 2011; Rasmusson et al., 2020). To avoid bias in our analyses caused by such affects, O_2 flux data associated with daytime temperatures above 28°C were excluded. This left 2,115 hours of data for analysis, distributed as 1,029 hours of daytime values and 1,086 h of nighttime values.

While the daytime O_2 fluxes reflect the net result of photosynthetic processes and respiration, the nighttime fluxes represent exclusively respiratory processes in the seagrass meadow. To identify trends in respiration, hourly O_2 flux measurements during the night were examined using a least-squares linear regression analysis of the binned values of fluxes with "time after dusk" as the independent variable. Nighttime hourly means for three other variables that could potentially impact O_2 flux variability were also examined: current velocity, O_2 concentration, and water temperature.

For current velocity, which has been shown to stimulate O_2 fluxes in seagrass meadows (Hume et al., 2011; Long et al., 2015), a two-sample t-test assuming unequal variances (alpha = 0.05, DF = 210) was performed comparing velocity measurements during the first hour of night and velocity measurements during the last hour of night. Mean hourly O_2 concentration values for the first and last hours of night were examined, and the change in O_2 concentration over the course of the night was evaluated. The O_2 concentrations were compared to those in the literature and assessed for their likelihood to impact respiration. To evaluate the role of temperature, which has been shown to impact respiration (Staehr & Borum, 2011), a first order Q10 model calculation was performed. This classic model (Berry and Raison, 1979), which expresses how much respiration will increase if the temperature increases by 10°C, was used to calculate the expected change in respiration through the night using a Q10 value for respiration in *Zostera marina* meadows of 2.4 reported by Marsh et al., 1986.

The linear decrease in the nighttime O_2 fluxes and a corresponding linear increase for daytime respiration combined with a fitted standard Photosynthesis-Irradiance (P-I) curve (Webb et al., 1974), was used to create a 24-hour O_2 flux model. Residuals were calculated between the measured and modeled hourly O_2 fluxes and used to evaluate the model.

Results

A linear fitting to the binned nighttime O_2 fluxes showed a clear trend (R^2: 0.946, slope: 5.11 mmol m^{-2} d^{-1} h^{-1}, p-value: $2.37*10^{-6}$) throughout the night (Figure 2A). A very similar trend (R^2: 0.027, slope: 5.09 mmol m^{-2} d^{-1} h^{-1}, p-value: $4.09*10^{-8}$) was obtained when fitting the un-binned O_2 flux data (not shown). These fits revealed that respiration in the seagrass meadow decreased by 29% through the night.

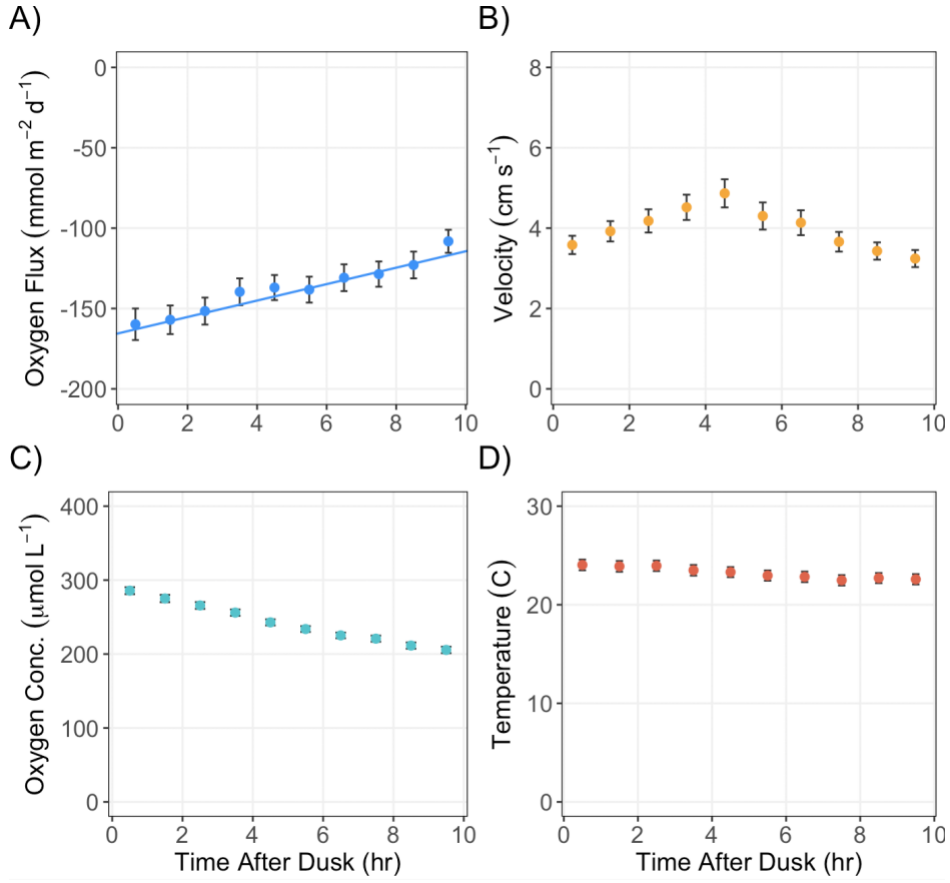

Figure 2. Average nighttime measurements of key variables from 1,086 h of nighttime data plotted against time after dusk. The first hour after dusk represents measurements between 20:00 and 21:00 and the last hour before dawn represents measurements between 05:00 and 06:00. A) The trend in the mean O_2 flux (R^2 = 0.95) and the mean measurements for each hour ± SE. B) Mean measurements of current velocity for each hour after dusk ± SE. C) Mean measurements of O_2 concentration for each hour after dusk ± SE. D) Mean measurements of water temperature for each hour after dusk ± SE.

The mean hourly velocity does show some variation after dusk, with a slight increase during the middle of the night (Figure 2B). However, during the first hour after dusk and the last hour before dawn the current velocities were not statistically different (p-value: 0.14), and thus, were

evaluated not to impact respiration significantly. Water column O_2 concentration decreased with time after dusk (Figure 2C) as benthic and water column respiration consumed oxygen, and amounted to a 28% decline over the course of the night. However, the O_2 level never fell below 200 μmol L^{-1}. Water column temperature (Figure 2D) declined throughout the night by 11%. The observed temperature change of 1.5°C, based on a Q10 value of 2.4, would only decrease metabolism by 2.0%, far from the identified 29% decline through the night.

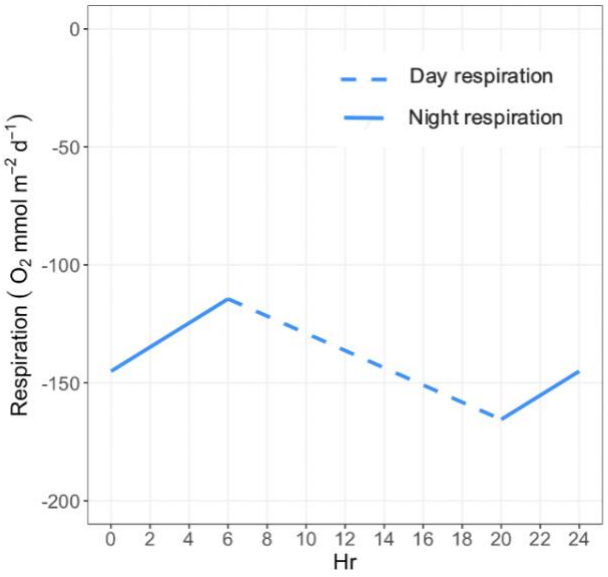

Figure 3. Respiration trend throughout the full 24-hour period, including the linear trend for respiration during the nighttime hours (20:00-06:00; same as in Figure 2A) and the theoretical daytime respiration.

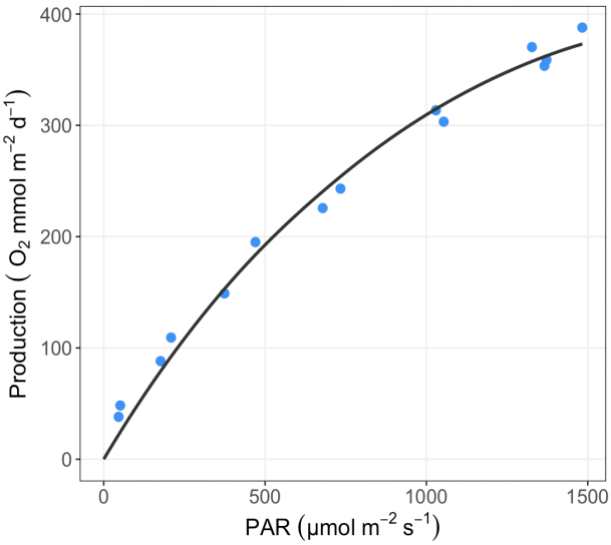

Figure 4. Photosynthesis-Irradiance (PI) curve with production values found from 1,029 of O_2 flux measurements. Production values are found by subtracting the theoretical daytime respiration (Figure 2) from the mean O_2 flux. The fitted PI curve has an R^2 of 0.989.

A piecewise linear description of night- and daytime respiration (Figure 3) combined with a widely used standard P-I curve (Figure 4) for daytime production (R^2 = 0.989) generated a model for hourly O_2 fluxes through the full 24-hour period. (Figure 5). This model approximation of the measured O_2 fluxes had an R^2 of 0.993. The hourly residuals had small discrepancies during the early morning and the late evening, but beyond that showed no general trend in variation over the course of the day. In the measured values for O_2 flux and PAR there is a slight decline during the middle of the day (Figure 5) which is likely due to pop-up storm formation that often occurs in the early afternoon at the site.

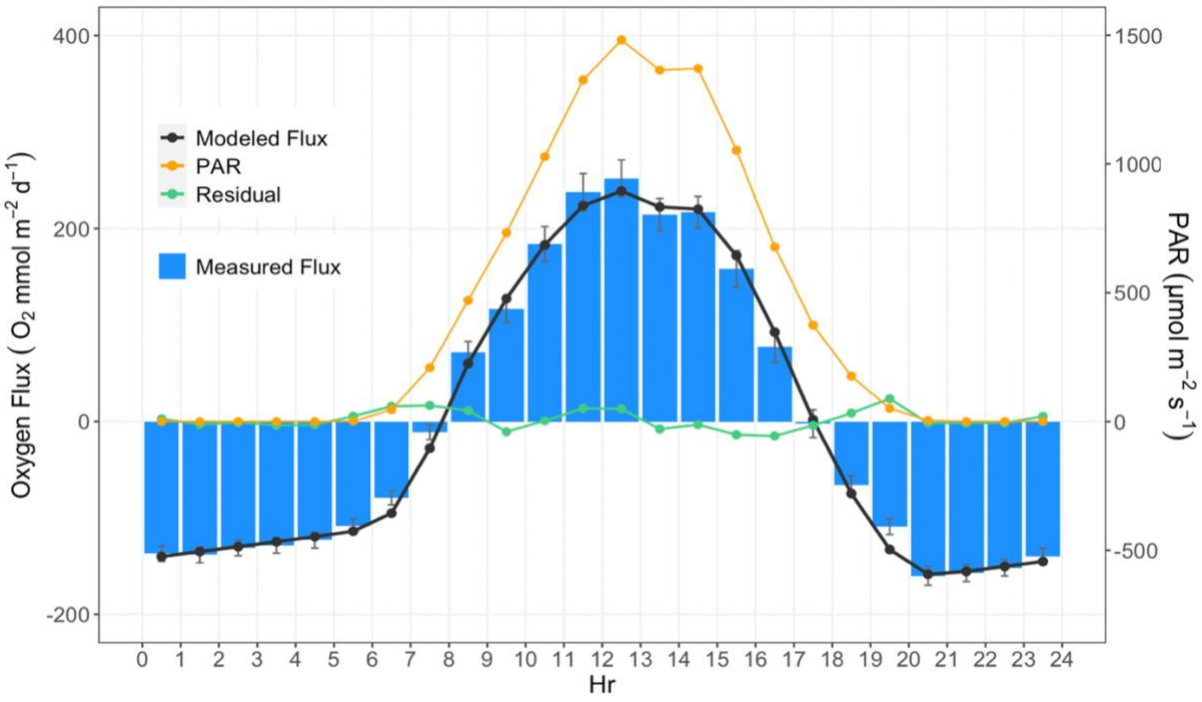

Figure 5. A model for O_2 flux over the full 24-hour period based on PAR (Figure 4) and the piecewise linear variation in respiration (Figure 3). The modeled O_2 flux has an R^2 of 0.993.

Discussion

Past studies have questioned key assumptions behind the conventional way metabolic numbers for aquatic ecosystems are calculated; that night- and daytime respiration are constant

and equal (Pringault et al., 2009; Sadro et al., 2014; Howarth et al., 2014; Mantikci et al., 2020). If these assumptions do not hold, errors will arise when using only a few hours of nighttime respiration measurements to represent respiration for a 24-hour period. The linear decrease in respiration by 29% through the night identified here for a temperate seagrass meadow (Figure 2A) showed that this concern is warranted.

Water column temperature and current velocity in our study did not show variation that could explain this significant decline in nighttime respiration. Water column O_2 concentrations decreased throughout the night by 28% due to respiratory activity in both the water column and the benthic substrate. Although O_2 concentrations stayed at levels that are usually considered well above respiration limiting conditions in seagrass meadows (Borum et al., 2005; Rasmusson et al., 2020), it is possible that aerobic respiration in the sediment would decrease as bottom water O_2 concentration declined (Thamdrup et al., 1998; Lehmann et al., 2009). However, as the sedimentary uptake of O_2 at this site has been measured to only be a small component of total O_2 fluxes (Hume et al., 2011; Rheuban et al., 2014a) and the majority of metabolic activity in the whole seagrass ecosystem is dominated by the plants themselves (Søndergaard, 1979; Colmer & Pedersen, 2008; Duarte et al., 2014) we assess that the decrease in sediment respiration triggered by decline in O_2 concentration would only explain a small fraction of the observed drop in whole ecosystem respiration throughout the night (Figure 2).

Consequently, we find it most likely that the variation in respiration over the full 24-hour period (Figure 3) is primarily caused by highly labile compounds produced by photosynthetic processes that accumulate as the day progresses and subsequently are consumed during the night; an explanation that is supported by several studies. For example, these compounds can be found on the surfaces of seagrass tissues in the form of dissolved organic carbon exudates (DOCs), which have been shown in laboratory experiments to stimulate respiration (Moriarty & Iverson, 1986) and decrease in concentration on seagrass leaves after the onset of darkness (Penhale & Smith, 1977). They have also been shown to stimulate microbial activity in the root zone of seagrasses (Gribsholt & Kristensen, 2002; Aoki & McGlathery, 2018) and are quickly consumed by epiphytic bacteria when exuded from seagrass leaf tissue (Kirchman et al., 1984). This explanation is further supported by the fact that the piecewise linear description of night- and daytime respiration (Figure 3) combined with a widely used P-I curve for daytime production generated an O_2 flux model that accurately predicted ($R^2 = 0.993$) the measured hourly fluxes

over a full 24-hour period (Figure 5). The modeled flux was able to better predict the lower measured fluxes in the afternoon at the same light levels compared to the observed morning measurements. In summary, our findings provide evidence, based on extensive metabolic aquatic eddy covariance measurements under naturally varying field conditions, for substantial variation in seagrass respiration which is likely due to the production and consumption of highly labile compounds over the diel cycle; a dynamic mechanism that has been hypothesized in previous studies (Howarth et al., 2014; Rheuban et al., 2014b; Attard & Glud, 2020).

The documented variation in respiration confirms that the widely used assumption in conventional calculations of daily GPP and R, that respiration stays constant during day and night, is not fulfilled (Figure 2A and Figure 3). However, if respiration varies linearly through night and day, as found to be a good approximation here and likely applicable to other phototrophic aquatic ecosystems, the separately integrated night- and daytime respiration are equal, which implies that the conventional means for calculating daily R and GPP still hold. However, it is important to have measurements throughout the night to get an accurate mean of nighttime respiration. Using, for example, only a few evening night hours would result in a significant overestimation of daily R.

As a concluding remark, our study exemplifies the high value of long-term research. Our metabolic findings wouldn't have been possible without the long-term dataset consisting of more than 2,100 hours of aquatic eddy covariance data collected at the VCR LTER site. The highly dynamic nature of seagrass metabolism would have made it difficult to see trends in parameters such as respiration. Future studies to further refine our understanding of respiration for seagrass meadows, and what drives it, should preferably include quantifications of above- and belowground dissolved organic carbon exudates in tandem with aquatic eddy covariance O_2 flux measurements. A more complete picture of seagrass metabolism and its drivers are critically important to correctly assess the role these threatened ecosystems play in climate change mitigation through coastal blue carbon sequestration and storage.

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Chapter 2: Non-dissolved Gas Fluxes in a Seagrass Meadow Measured with a Novel, Automated Bubble Trap

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This chapter will likely be submitted for publication to L&O or L&O Methods

Abstract

Dissolved oxygen (O_2) flux measurements have been used to quantify benthic metabolism, describe carbon cycling, and assess ecosystem health in aquatic ecosystems. Ebullition, the release of non-dissolved gases, has been observed in many of these systems, and it has been suggested that O_2 bubbles could represent a significant part of whole benthic ecosystem O_2 flux. Previous studies have examined ebullition in seagrass meadows over very short time periods and with coarse temporal resolution. In this study we introduce a novel, automated bubble trap design that measures non-dissolved gas fluxes at a fine temporal scale (10 min). With this new method, we examined: 1. the role of environmental variables in controlling ebullition rates, 2. the size of the non-dissolved O_2 flux component in the total O_2 fluxes, and 3. the non-dissolved fluxes of CO_2 and CH_4, both important greenhouse gases, in temperate seagrass meadows. For restored *Zostera marina* meadows in Virginia's coastal bays, we found that the O_2 bubble flux makes up a very small percentage of total O_2 fluxes, only increasing Gross Primary Productivity estimates by 2.4% in July, 3.6% in August. By comparing 10-minute bubble fluxes to environmental variables, we found that high light intensities, supersaturated O_2 conditions, and low tides stimulate ebullition. Observations of bubbles on abiotic surfaces indicate bubble formation may not be representative of on-site photosynthesis, a question that needs further study.

Introduction

'Blue carbon' is the term used to describe carbon that is sequestered and stored in vegetated coastal ecosystems, including in seagrass meadows (Murray et al., 2011; Nellemann et al., 2009). These blue carbon ecosystems are viewed as an important nature-based solution to reduce global atmospheric carbon dioxide concentrations and mitigate climate change (Duarte et al., 2013). Globally, seagrass meadows alone store between 4,200 and 8,400 Tg of organic carbon in their sediments and 151 Tg of organic carbon in biomass (Fourqurean et al., 2012). To describe in situ carbon sequestration in these ecosystems, we need accurate assessments of daily

metabolic values: Gross Primary Productivity (GPP), Respiration (R), and Net Ecosystem Metabolism (NEM), all of which can be estimated from benthic oxygen (O_2) fluxes (Odum, 1956; Berger et al., 2020). These O_2 fluxes usually only include dissolved O_2 fluxes and exclude ebullition (Berg et al., 2003; Glud, 2008; Middleburg et al., 2005).

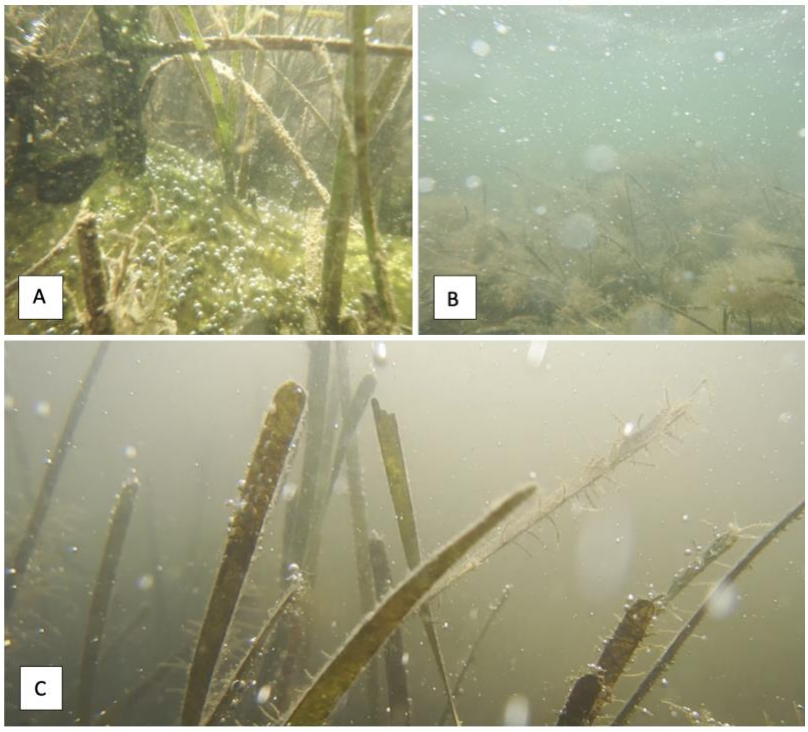

Figure 1. A) Bubble formation on algal mat growing on sediment surface in a *Zostera marina* meadow. B) Bubbles in the water column above the seagrass canopy. C) Bubbles formed on the seagrass leaves and epiphytes in the meadow (pictures taken by Sarah Cox, Berg lab REU at the VCR LTER Summer 2019)

In seagrasses, substantial bubble production has been observed (Figure 1) during periods of high productivity (Wilson et al., 2012), indicating that non-dissolved O_2 could represent a significant product of photosynthesis. This suggests that substantial O_2 fluxes may be occurring in the form of ebullition, and thus, are not accounted for in traditional calculations of seagrass ecosystem GPP, R, and NEM from dissolved O_2 fluxes (Borum et al., 2007; Long et al., 2019). In Virginia's coastal bays, dissolved O_2 flux measurements have been used to develop a detailed understanding of the metabolism and health of the local temperate seagrass ecosystem (Berger et al., 2020; Hume et al., 2011; Rheuban et al., 2014a; Rheuban et al., 2014b).

Quantifying non-dissolved gas fluxes is also important to estimate carbon emissions in blue carbon ecosystems (Oreska et al., 2020; Rosentreter et al., 2021), as ebullition is a significant form of flux for methane (CH_4), a powerful greenhouse gas (Hornafius et al., 1999; Keller & Stallard, 1994; Walter et al., 2007). Carbon dioxide (CO_2) bubble emissions have been

examined in some vegetated aquatic ecosystems (Capooci et al., 2019; Diefenderfer et al., 2018), but remain largely unstudied in seagrass meadows. Ebullition of these gases and O_2 in aquatic ecosystems is highly dynamic and varies considerably under different conditions. Specifically, gas fluxes can vary based on changing temperature and atmospheric pressure (Kellner et al., 2006), water depth (Chanton et al., 1989; Klotz, 1963), and light availability (Long et al., 2019; Lyimo et al., 2018). To enhance our understanding of non-dissolved gases fluxes of O_2, CH_4, and CO_2 in seagrass meadows, we need to have more precise measurements of ebullition.

Ebullition in aquatic systems is typically measured using floating bubble traps which consists of an inverted funnel with a gas collection chamber in which gas volume is measured and can be collected for analysis (Casper et al., 2000; Cheng et al., 2014; Keller & Stallard, 1994). These bubble traps are typically sampled manually a few times a day and are deployed for a short period of time, with studies typically spanning a few weeks or a few days (Cheng et al., 2014; Keller & Stallard, 1994; Long et al., 2019; Varadharajan et al., 2010). Our study uses a novel, automated bubble trap design that can make frequent measurements of gas volume in the collection chamber, allowing us to quantify ebullition rates in aquatic ecosystems at a fine temporal scale, and thus, examine the effects of quickly changing environmental conditions. The automated aspect of this design allows the traps to be deployed for multiple days at a time. To our knowledge, this will be the first time a bubble trap of this type will have been used in a coastal ecosystem to measure non-dissolved gas ebullition rates.

Methods

Site description

All data were collected in a temperate seagrass meadow in the shallow coastal bays of the Delmarva peninsula in Virginia. These bays are part of the Virginia Coast Reserve Long-Term Ecological Research site (VCR LTER) and are home to the largest restored eelgrass (*Zostera marina*) meadows in the world (Orth & McGlathery, 2012). Our study sites were located in South Bay, a subtidal lagoon bordered by barrier islands to the east and the Delmarva peninsula coast to the west, and connected to the Atlantic by two inlets (Berger et al., 2020). Within South Bay, a site in the center of the meadow (SB152) was selected as the primary location for the study. In July, a second site (North) was added for comparison that was located along the

northern edge of the South Bay seagrass meadow. This site was deeper and was located closer to the inlet (Figure 2).

Figure 2. The VCR LTER South Bay seagrass meadow located off the east coast of the Delmarva peninsula. This map shows the original seeding plots for the meadow restoration, as well as the extent of the meadow in South Bay as of 2016 (modified from Oreska et al. 2020). The two sites (SB152 and North) for this study are marked on the map in orange.

Bubble trap design

This study developed and tested a novel bubble trap design that samples cumulative collected gas volume by logging pressure changes in the gas collection chamber. The technique monitors changes in volume at a fine temporal resolution, allowing for comparison with rapidly changing environmental variables. The new design was derived from systems used in freshwater ecosystems (Maher et al., 2019; Varadharajan et al., 2010), but was simplified and adapted for use in coastal environments. The design uses off-the-shelf loggers rather than manually programmed systems for each bubble trap and measures benthic ebullition at a fixed depth instead of at the air-water interface.

Our bubble trap design (Figure 3) consists of three major components: the pressure logger housing, the gas collection chamber, and the funnel, which were mounted onto PVC structures that were affixed in the sediment. The clear funnel is made of two parts: a white 12.7 cm diameter polyethylene funnel and a clear 0.3 cm thick PVC film skirt, glued together to create a smooth, continuous surface inside the funnel. The circular bottom of the funnel is 30 cm in diameter, creating a sampling area of 706 cm^2 on the benthic surface, suspended at the top of the seagrass canopy at 30 cm above the sediment. The spout of the funnel is connected to the gas collection chamber with a rubber stopper, and is removable from the collection chamber, primarily for cleaning of any biofouling.

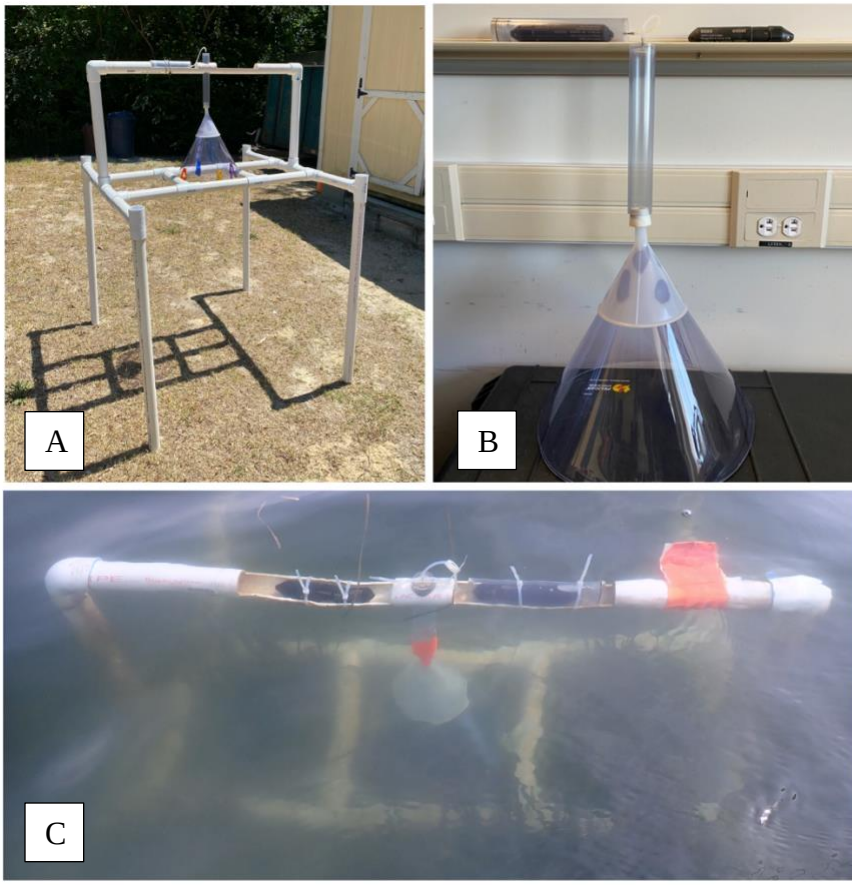

Figure 3. A) The bubble trap design mounted onto a PVC structure on land. The legs of the PVC structure were pushed into the sediment until the bottom of the funnel was ~30cm above the sediment surface. B) All components of the bubble trap system. The funnel is 30 cm in diameter at the downward facing opening and is connected to the collection chamber above. The top of the collection chamber connects via plastic tubing to the logger in watertight housing. C) One bubble trap system deployed in the meadow. The reference logger is positioned approximately at the same water depth as the logger in the housing.

The gas collection chamber (Figure 3B) is made of clear PVC plastic pipe that has an inside diameter of 2.67 cm and a height of 21.59 cm, and can hold 120.34 mL of gas. The gas collection chamber has a thin stainless-steel pipe (ID 0.13 cm) located at the center of the plastic cap. Plastic Tygon tubing (ID 0.09 cm) is attached to this stainless-steel pipe, and connects to

another stainless steel pipe of the same diameter that is set in the logger housing. Due to the small size of the tubing, bubbles would occasionally become stuck, biasing readings around low tide. This was solved with a tubing of a larger size (see Supplementary Materials). The logger housing is made of clear PVC plastic pipe that has an inside diameter of 3.50 cm and is 16.51 cm in length in the inside opening. This design is meant to provide just enough space for the logger (3.18 cm in diameter and 15.24 cm in length) to fit inside the housing. The end of the logger housing with the stainless-steel tube is sealed permanently, while the other end has an airtight screw cap sealed with an O-ring, which allows the logger to be easily removed from the housing for data download. Prior to deployment, the logger housing and the thin plastic tubing connecting the housing to the collection chamber were filled with deionized water.

The pressure logger used for this study is the ONSET HOBO U-20L-04 logger, which records absolute pressure using a ceramic sensor, and logs temperature as well. The loggers were set to record both pressure and temperature every 30 seconds. Because the logger records absolute pressure, a reference logger is placed on the PVC structure at approximately the same depth as the logger housing. ONSET specifies that the HOBO U-20L-04 loggers all come calibrated from the factory with a resolution of <0.14cm of water level and a typical error for water level accuracy of ± 0.1% FS.

Volume Calculation

Due to variability in 30-second water depth above the bubble traps caused by wave activity in South Bay, the 30-second measurements of pressure and temperature were averaged to 10-minute values. The reference logger's measured absolute pressure was subtracted from the absolute pressure of the logger in the housing, to account for tidal changes in water depth and changes in atmospheric pressure. This gives the differential pressure, which is directly proportional to the height of the gas volume in the collection chamber. To find the volume from differential pressure, a relationship between pressure and gas volume is used given by a conversion from kPa to millimeters of water (Eq. 1):

$$\Delta P * \alpha = h \qquad \text{(Eq. 1)}$$

Where ΔP is the differential pressure (kPa) between the reference logger and the logger in the housing, α (mm kPa^{-1}) is a constant at a given water density, and h is the height of displaced water (mm) in the collection chamber. Using the dimensions of the collection chamber, the

collected gas volume is calculated from h. The constant α varies depending on density, which is affected by both water temperature and salinity. For South Bay, due to the absence of riverine inputs, salinity stays relatively constant (Berg et al., 2019; Oreska et al., 2021) with an average June salinity of 30.3 ppt (Berg et al., 2019). This average was used to determine α along with the measured variable temperature for each 10-minute period. At 20°C, the value for α would be 102.42 mm kPa^{-1}.

To convert the volume readings to a standard temperature and standard pressure to compare bubble volumes collected on different days, times, and different environmental conditions, the ideal gas law equation was used:

$$PV = nRT \quad \text{(Eq. 2)}$$

Where P is the pressure in pascals (Pa), V is volume in cubic meters, n is the number of moles of gas, R is the gas constant (8.31441 J K^{-1} mol^{-1}), and T is temperature in Kelvin (K). With measured P and T, and the calculated value for V, n can be estimated. Using n, V can be calculated for a standard T and P. Other studies with pressure sensor bubble traps have converted their volume measurements similarly, and used a standard temperature of 20°C and pressure of 1 atm for comparison (Varadharajan et al., 2010). Data collected in the summer of 2019 at our primary site (SB152) shows that summer temperatures range from 21.1°C to 33.2°C, with an average value of 26.5°C. Due to this, the corrected volume was standardized here to a temperature of 25°C and a standard pressure of 1 atm, allowing for comparison across different days and times.

Method Testing

The novel bubble trap design was tested for accuracy first in the lab and then in the field by bubbling known volumes of gas into the collection chamber and comparing those to calculated values from the pressure readings as outlined above. In a water filled tank and using plastic tubing and a syringe, known amounts of gas were injected into the submerged collection chamber. After each addition, the system was left to equilibrate for 5 or 10 minutes. Pressure readings were averaged for each known volume (a 5- or 10-minute period), and the differential pressure between the loggers was found and a volume was calculated.

In the field, a similar experiment was performed with the exact setup as used for field deployments. The logger housing containing the trap logger, the gas collection chamber with the

funnel attached, and the reference logger were mounted onto the submerged PVC structure (Figure 3) at the central site in South Bay (SB152). Plastic tubing was fed under the funnel and known amounts of gas were bubbled in using a syringe. After each bubble addition, the system was left to equilibrate for 10 minutes. The volume was calculated as described above. The calculated volume was plotted and compared against the known injected gas volume.

Field Sampling

Field sampling was conducted from early June through mid-August in 2021 using five bubble trap systems in South Bay. During the sampling period the bubble traps were typically deployed for 2 days at a time, with some variability due to weather and boat availability. The PVC structures were inserted into the sediment at the beginning of the summer, and remained in the field throughout the season, occasionally being cleared of biofouling with a coarse brush. The logger housing, with the logger inside, and plastic tubing that connected the housing and the collection chamber would be filled with deionized water at the beginning of each deployment. At the end of each deployment, gas was emptied from the collection chambers and any biofouling was removed for all deployed bubble traps with a brush. The surfaces of the funnel and collection chamber were also cleaned with a 10% bleach solution every few deployments or more frequently if biofouling was visible to the naked eye. The loggers were returned to the lab every few deployments for data offloading.

Gas samples was collected from each bubble trap for later analysis at the end of some deployments using a syringe inserted into the thin tubing at the top of the collection chamber. The gas was immediately injected into an exetainer filled with 12 ml of N_2 and 0.2 ml 0.01 M $ZnC_4H_6O_4$ to prevent microbial activity. Fluxes of gases were derived from % volume measurements along with manual gas volume sampling and sampling area. Gas sample O_2 content was determined using a fixed needle O_2 optode and meter (Pyroscience). Gas sample CH_4 and CO_2 concentrations were analyzed on a Varian 450-Gas Chromatograph. The R^2 values for the standard curves ranged from 0.989 to 0.999.

During the deployments, several environmental variables were measured in tandem with the bubble trap measurements. Readings taken every 30 seconds from the reference HOBO U-20L-04 (ONSET) logger were used to estimate relative water depth from pressure changes and directly measure water temperature. Photosynthetically Active Radiation (PAR) was measured

every 5 minutes using submersible planar 2π Odyssey PAR sensors (Dataflow 199 Systems) and O_2 concentration, O_2 saturation, and an additional temperature reading were measured every 10 minutes using submersible O_2 optodes (miniDOT, PME). For some deployments, an acoustic Doppler velocimeter (a Vector from Nortek-AS) mounted on a steel frame was used to measure 3D current velocity at a sampling speed of 1Hz. Every two weeks, mean seagrass shoot density and shoot length was estimated by counting individual shoots in situ from 10 quadrats (0.25 m^2 each) and measuring the longest shoot length in each quadrat.

In exploratory fieldwork prior to this study, bubble formation was observed in the seagrass meadows on abiotic surfaces, providing some indication that bubbles on seagrass leaves do not necessarily represent on-site photosynthesis. To examine bubble formation, 2 of the 5 bubble traps were temporarily deployed over artificial seagrass mats (Figure 4). This experiment occurred in mid-July for 10 days at SB152. The artificial seagrass leaves were made from polypropylene film, approximately 8 mm wide (Sarah Sojka, pers. commun., 2020). Four leaves were bound together to form a shoot and attached to a wooden dowel that was strapped into a net stretched across a meter square PVC frame. The artificial seagrass beds had 250 shoots per m^2.

Figure 4. The artificial seagrass mats were deployed under two bubble traps in tandem with three traps sampling concurrently over natural seagrass. The mats were a square meter in size, and the seagrass leaves were made from polypropylene film.

The artificial mats were placed underneath of the bubble traps in a meter square area of bare sediment that had been cleared of vegetation, and the gas volume collected was assessed in the

same way as for the seagrass meadow, described above. Following this experiment and the removal of the artificial seagrass mats, the 2 bubble traps were left to sample bare sediment for a week to provide a reference point for comparison.

Statistical analysis

A linear regression was performed to compare known volumes and calculated volumes for accuracy tests, with the slopes compared to 1. Separate regressions were performed for the summary of the laboratory experiments and the summary of the field experiments. Volume readings for each trap in every deployment was visually evaluated and anomalies were removed (see Supplementary Materials). The mean volume across traps was calculated to account for system variability and to give a representative value for gas flux at that site. From the cumulative volume readings, a mean 10-minute trap ebullition rate was derived. These gas flux rates were then binned and compared against environmental variables. For PAR, O_2 saturation, and depth, linear regressions were performed for binned and un-binned data. To examine the trend of gas flux over the course of the day, binned hourly gas flux was plotted against the hour of day, and a smoothing curve was applied using the "loess" function in geom_smooth in R (Wickham, 2009). A multiple linear regression was conducted with 10-minute trap gas flux as the dependent variable and PAR, O_2 saturation, O_2 concentration, depth, water temperature, current velocity, and seagrass shoot density as independent variables.

Mean 10-minute gas flux was compared between the two sites and over the course of the summer. This was done using a two-sample independent t-test assuming unequal variance. A significance level of 0.05 was used for all statistical tests. For the artificial seagrass mat and bare sediment field experiments, a two-sample independent t-test assuming unequal variance was also used to compare between the ebullition rates for the experimental treatment traps and the control traps over natural seagrass. To analyze gas composition variation spatially, the gas % volume measured for O_2, CO_2, and CH_4 and the calculated flux of each gas were compared between sites using two-sample independent t-tests assuming unequal variance. All shown error bars represent standard errors.

Results

Lab and field tests where known amounts of gas where bubbled into the collection chamber showed good agreement between the known volume and the calculated volume from the pressure readings (Figure 5). A linear regression for the lab data gave a slope of 0.96, a highly significant p-value of $<2*10^{-16}$, and a R^2 of 0.993 with 129 degrees of freedom. A similar regression for the field data gave a slope of 0.98, a highly significant p-value of $<2*10^{-16}$, and a R^2 of 0.977 with 20 degrees of freedom.

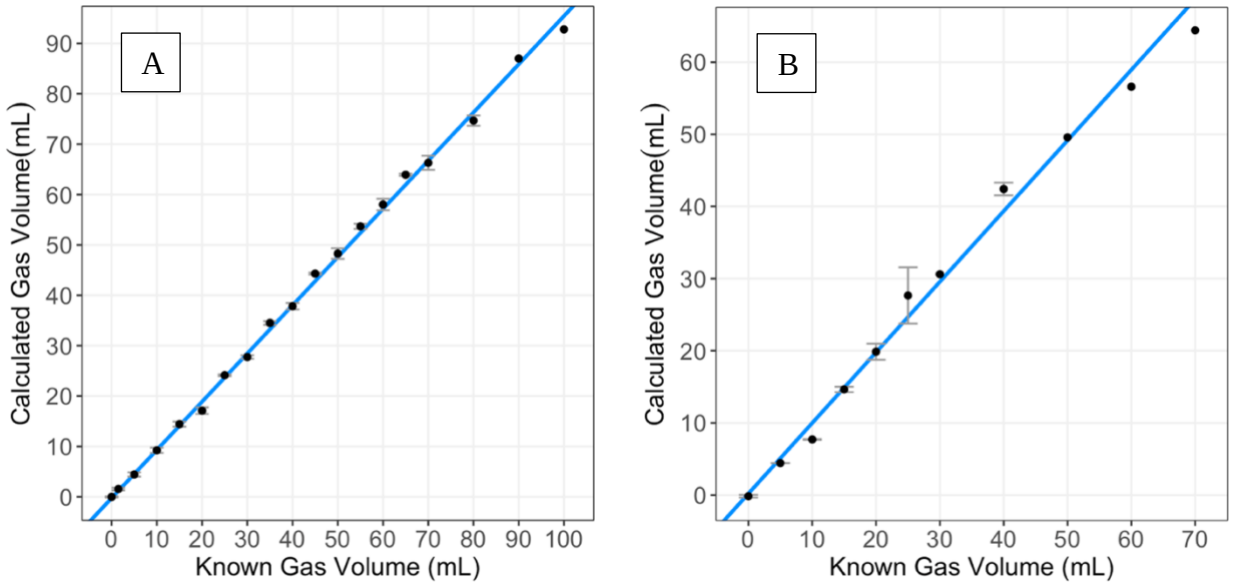

Figure 5. A) Binned values of calculated gas volume ± SE (n = mean: 6.9, max: 14, min: 1) for the bubbled known volume values in the laboratory tests. The linear regression results with the un-binned data show a slope of 0.96 and an R^2 of 0.993 with 129 degrees of freedom. The p-value of the regression was $<2*10^{-16}$. B) The mean values of calculated gas volume ± SE (n = mean: 2, max: 4, min: 1) for the bubbled known volume values in the field tests. The linear regression results with the un-binned data show a slope of 0.98 and an R^2 of 0.977 with 20 degrees of freedom. The p-value of the regression was $<2*10^{-16}$.

The volume readings at low tides showed an anomaly in some deployments, which were omitted for all further analyses. Laboratory and field testing indicated that this was due to air bubbles getting trapped in the thin tubing (ID 0.09 cm) during falling tide events that biased the pressure reading in the collection chamber. A modified system with thicker tubing (ID 0.39 cm) prevented this from happening as shown in laboratory and field tests (see Supplementary Materials).

Individual point-in-time volume readings from separate bubble traps showed variability, but averaging the 10-minute readings across traps gave a mean cumulative volume that showed a steady trend over time during deployments. Figure 6 shows an example deployment, including one trap's absolute pressure measurements for both loggers, as well as the cumulative volume readings for the three traps that were deployed.

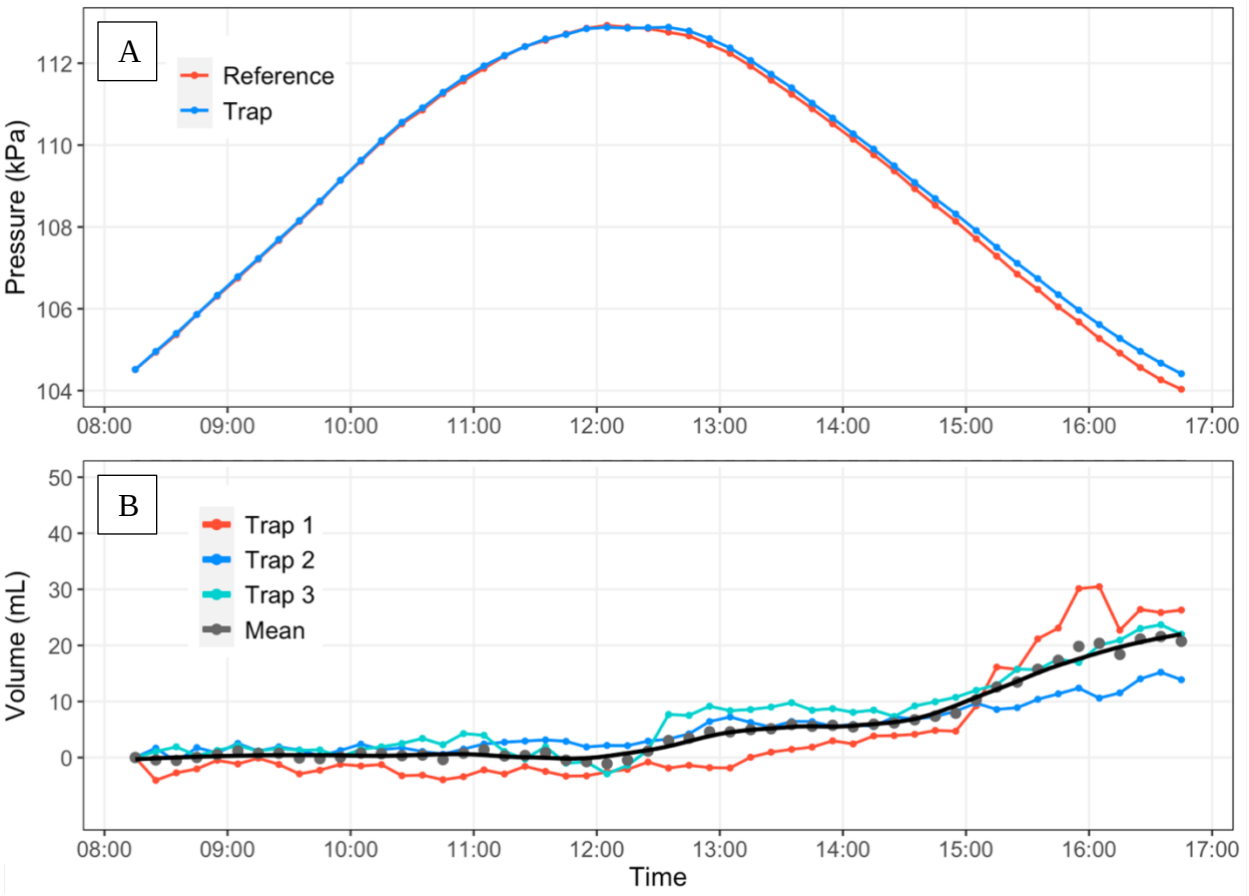

Figure 6. A) Absolute pressure readings from the reference pressure logger and the trap pressure logger (in the housing attached to the collection chamber) for trap 3 from an example deployment (July 14th, 08:15 to 16:45) from the central site (SB152). Tidal changes dominate the pressure signal, but as gas collects after noon the trap readings become greater than the reference readings. B) Individual trap values for the same example deployment showing readings for cumulative volume and the mean volume averaged across all three traps. A smoothing function was applied to the mean values to show the trend in volume over the course of the deployment.

In Figure 7, the mean volume values from the same example deployment shown in Figure 6 are plotted at the top, with water depth, water temperature, O_2 saturation, PAR, and current velocity shown as well. The current velocity measurements were set to start after the research

boat had left the site, which is why they do not encompass the very beginning of the bubble trap deployment. In this example deployment, the cumulative volume stays at zero from the early morning through noon. After noon, the volume starts to increase as ebullition occurs in the early afternoon, increasing further after 15:00. The volume stabilizes and stops increasing following 16:00. PAR, O_2 saturation, and water temperature all increase over the course of the day, with peaks in the late afternoon. PAR starts to decrease after 15:00, and O_2 saturation slightly starts to decrease after 16:00, when the cumulative volume stabilizes.

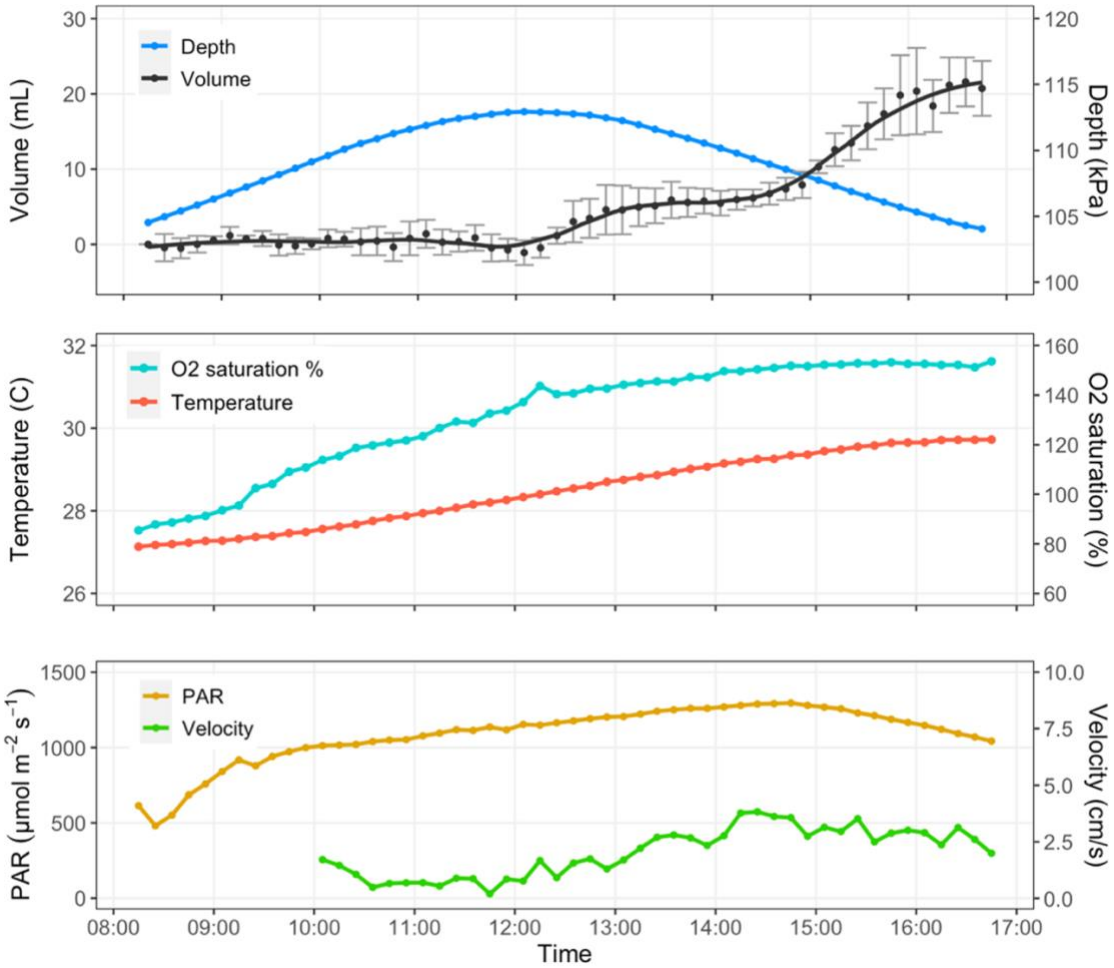

Figure 7. Example deployment (July 14th, 08:15 to 16:45) from the central site (SB152). The top panel shows the mean cumulative volume ± SE (n = 3) averaged across traps, with a smoothing function applied to show the trend in volume over the course of the deployment. The top panel also includes depth (measured as pressure). The middle panel shows O_2 saturation and water temperature, and the bottom panel shows PAR and current velocity.

It was possible to relate the rate of ebullition with environmental variables once 10-minute fluxes were calculated from cumulative volume measurements. For this comparison, only data from site SB152 was used. Binned values of mean trap gas flux had a positive relationship with PAR (Figure 8), and a linear regression gave a p-value of 0.00038 with a R^2 of 0.77 (DF of 9). For comparison, the un-binned mean trap gas flux and PAR linear regression gave a p-value of $1.24*10^{-11}$ and a R^2 of 0.03 (DF of 1455). Binned values of mean trap gas flux had a positive relationship with O_2 saturation (Figure 9), and a linear regression gave a p-value of 0.00004 with a R^2 of 0.66 (DF of 16). For comparison, the un-binned mean trap gas flux and O_2 saturation linear regression gave a p-value of $7.39*10^{-8}$ and a R^2 of 0.02 (DF of 1455). Binned values of mean trap gas flux had a positive relationship with depth (Figure 10), and a linear regression gave a p-value of 0.002 with a R^2 of 0.45 (DF of 16). For comparison, the un-binned mean trap gas flux and depth linear regression gave a p-value of $7.81*10^{-5}$ and a R^2 of 0.01 (DF of 1528).

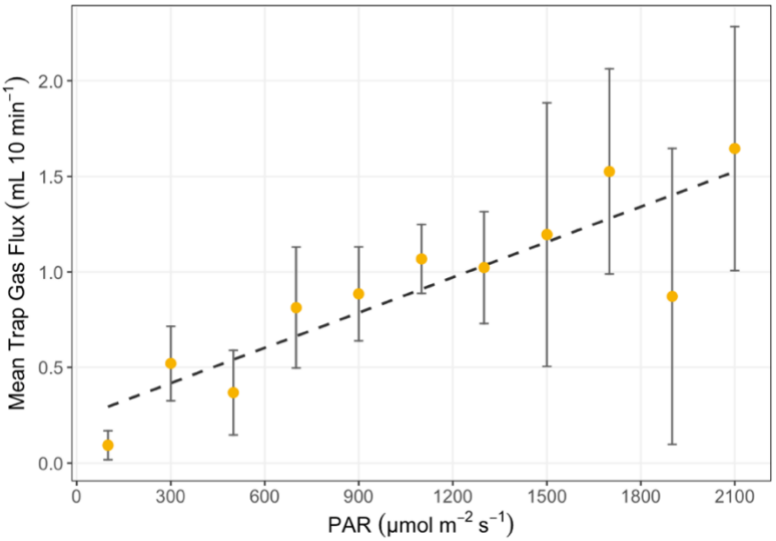

Figure 8. Binned mean 10-minute trap gas flux ± SE (n = mean: 127.5, max: 674, min: 5) compared to PAR values. The dashed line shows a linear fit for the binned values (p-value: 0.00038, R^2: 0.7710).

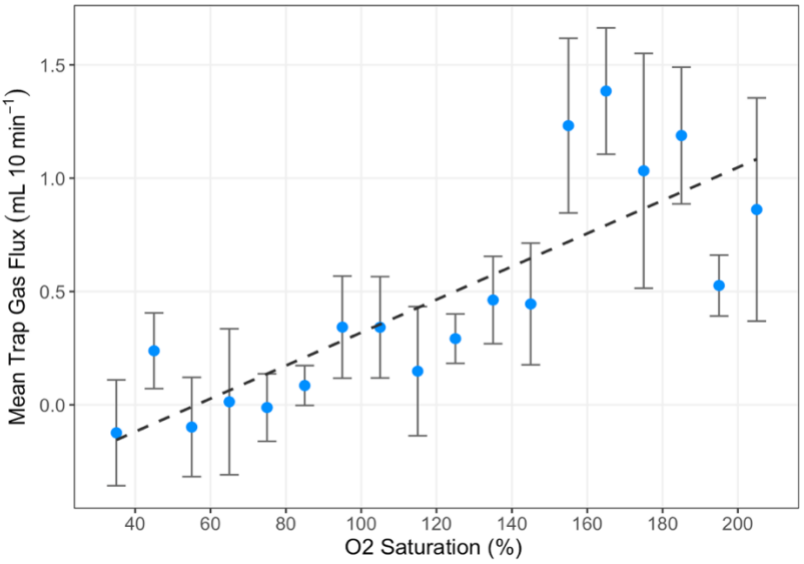

Figure 9. Binned mean 10-minute trap gas flux ± SE (n = mean: 80.5, max: 164, min: 10) compared to O_2 saturation values. The dashed line shows a linear fit for the binned values (p-value: 0.00004, R^2: 0.66).

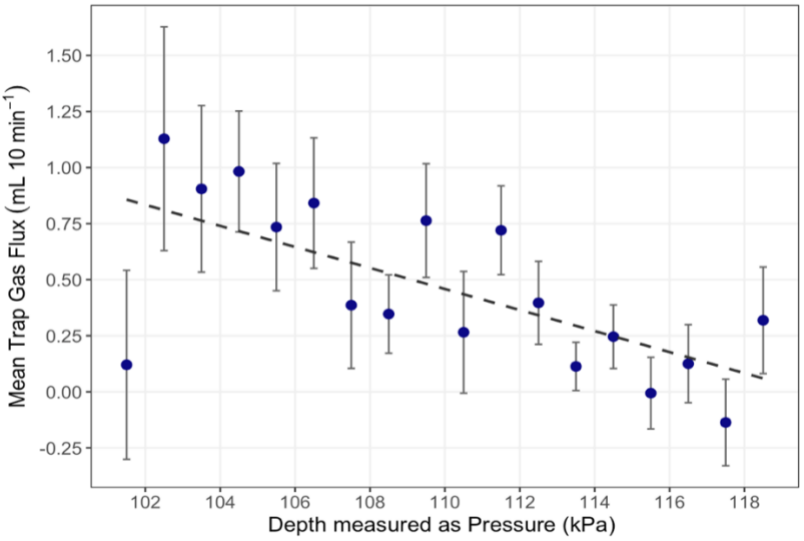

Figure 10. Binned mean 10-minute trap gas flux ± SE (n = mean: 85, max: 175, min: 10) compared to depth values, which are measured as pressure readings on the reference logger. The dashed line shows a linear fit for the binned values (p-value: 0.002, R^2: 0.45)

Ebullition is highest in the afternoon, from the hours of 13:00 to 18:00 (Figure 11). Plotting mean hourly PAR and O_2 saturation values, PAR peaks from the hours of 11:00 to 15:00 and O_2 saturation reaches close to its peak at 15:00 and only starts to decrease past 19:00 (Figure 11). The combination of the timing of these variables, coincides with the high afternoon ebullition rates.

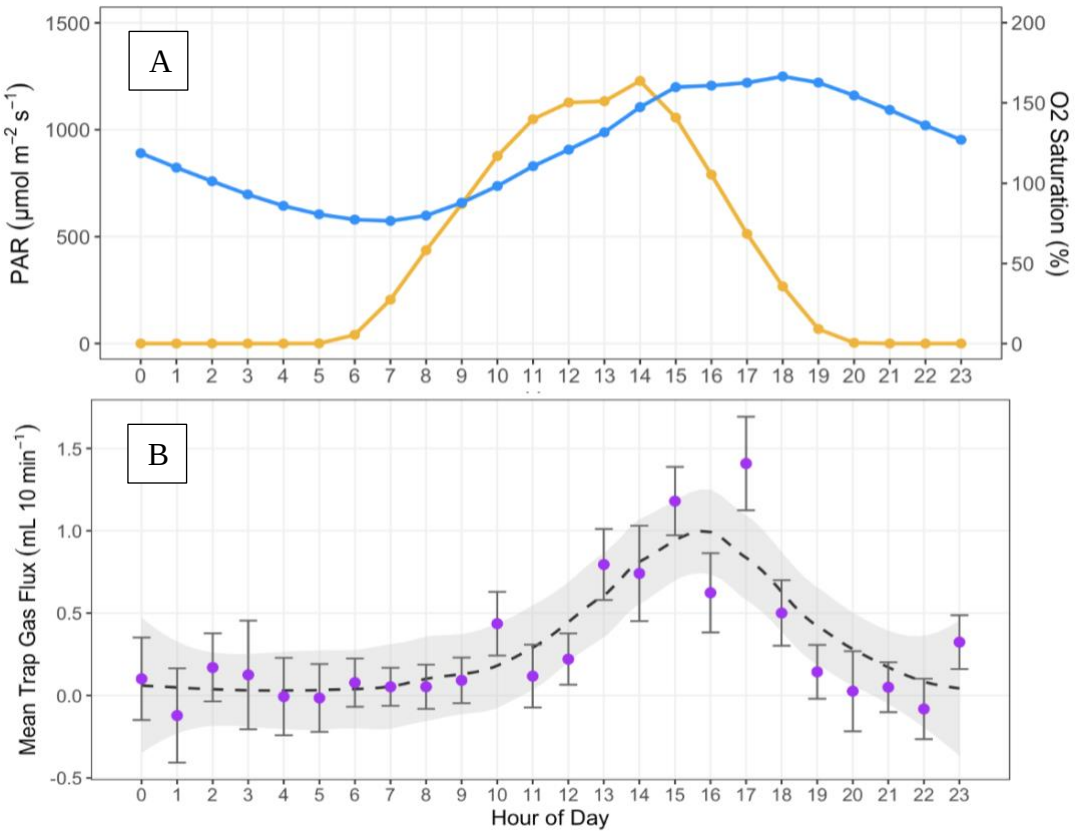

Figure 11. A) PAR and O_2 saturation means from the whole summer for each hour of day. B) Mean 10-minute trap gas flux values ± SE (n = mean: 63.8, max: 106, min: 16) binned for each hour of day. The fitting function (R, geom_smooth, "loess" method (Wickham, 2009)) is shown as a dashed line, with the SE of the fit shown in gray.

A multiple linear regression was performed using all data from SB152 to create a model for predicting 10-minute trap gas fluxes. The model used all measured variables during the study as independent variables: PAR, O_2 saturation, O_2 concentration, depth, water temperature, current velocity, and seagrass shoot density. The results of this multiple linear regression only gave an R^2 value of 0.073, indicating that the model including all variables only explained 7.3% of the variation in 10-minute gas fluxes. Although the p-value was highly significant ($3.875*10^{-15}$), the model was not a good predictor of bubble fluxes. After accounting for multicollinearity, the significant variables were PAR (p-value $1.11*10^{-6}$), O_2 saturation (p-value $1.09*10^{-5}$), depth (p-value 0.00047), and seagrass shoot density (p-value 0.00223).

An independent two sample t-test comparing data between sites (excluding June measurements, due to lack of sampling at the northern site at this time) resulted in a p-value of 0.0003811 (t = -3.5601, DF = 1669). At both SB152 and North, the difference between the mean

gas flux between July and August was not significant (SB152: p-value = 0.06284, t = 1.8622, DF = 1085; North: p-value = 0.2148, t = 1.2419, DF = 537). However, the difference between June and July at SB152 was significant (p-value: 0.0008259, t = 3.363, DF = 537). Mean trap ebullition was over two times higher in June than July and over four times higher in June than August at the central site, SB152 (Figure 12).

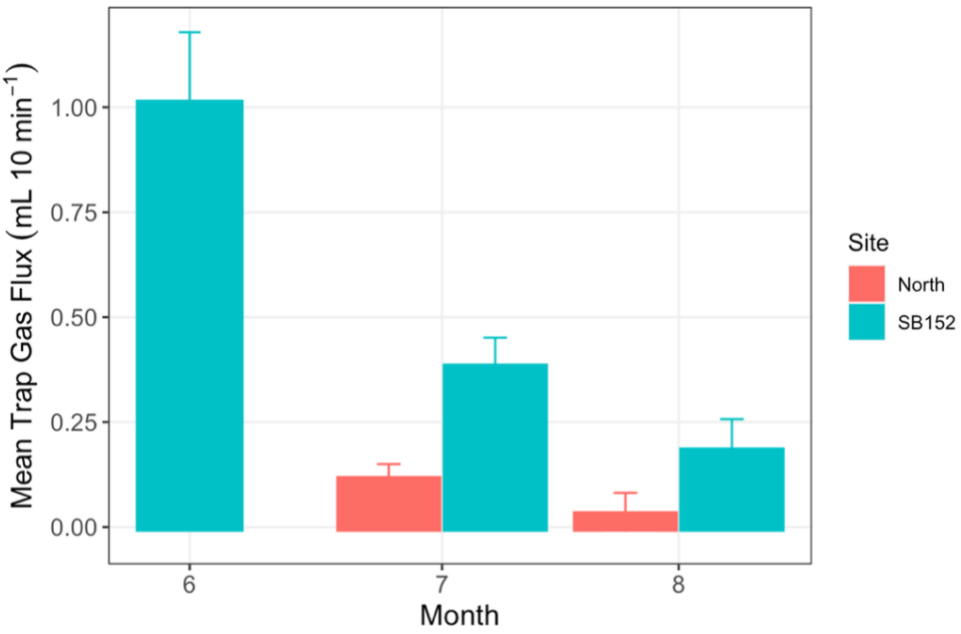

Figure 12. The mean 10-minute trap gas flux values ± SE (n = mean: 416.6, max: 640, min: 150) shown for each summer month and each site. The North site was at the northern edge of the South Bay meadow, while SB152 is in the very center of the meadow. Data at the North site was only collected in July and August, while sampling at SB152 went from June to July.

The apparent decrease over the course of the summer in ebullition rate could be attributed to a decrease in PAR and O_2 saturation that was also observed over the course of the summer (Figure 13). In previous results, a significant relationship between each of these variables and the 10-minute mean trap gas flux was shown (Figures 8 and 9). To compare across months, only data from SB152 was used. The results of an independent two sample t-test show that the difference in PAR was not significant between July and August (p-value = 0.1268, t = 1.5274, DF = 2363), but was significant between June and July (p-value = 0.002864, t = 2.9968, DF = 497). T-tests showed that O_2 saturation was significantly different between June and July (p-value = $2.865*10^{-6}$, t = 4.7222, DF = 644) and July and August (p-value = $8.016*10^{-13}$, t = 7.1994, DF = 2443) at SB152. PAR and O_2 saturation also varied significantly between the two sites (PAR: p-value =

<2.2*10^{-16}, t = -12.496, DF = 3608; O_2 saturation: p-value = <2.2*10^{-16}, t = -13.596, DF = 3433). Seagrass shoot density and leaf length varied between the North site and SB152. Shoot density was 34% lower at the North site than at SB152 and leaf length was 24% higher at the North site than at SB152.

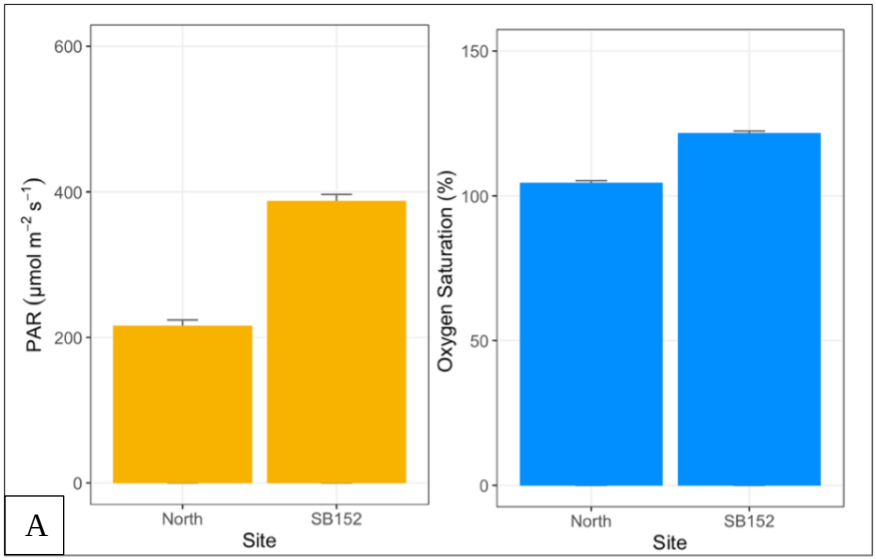

Figure 13. A) Mean PAR ± SE (n = North: 1207, SB152: 2868) on the left and O_2 saturation ± SE (n = North: 1207, SB152: 2868) on the right shown for each site. Just July and August data from both sites was included for this comparison as the North site was not sampled in June.

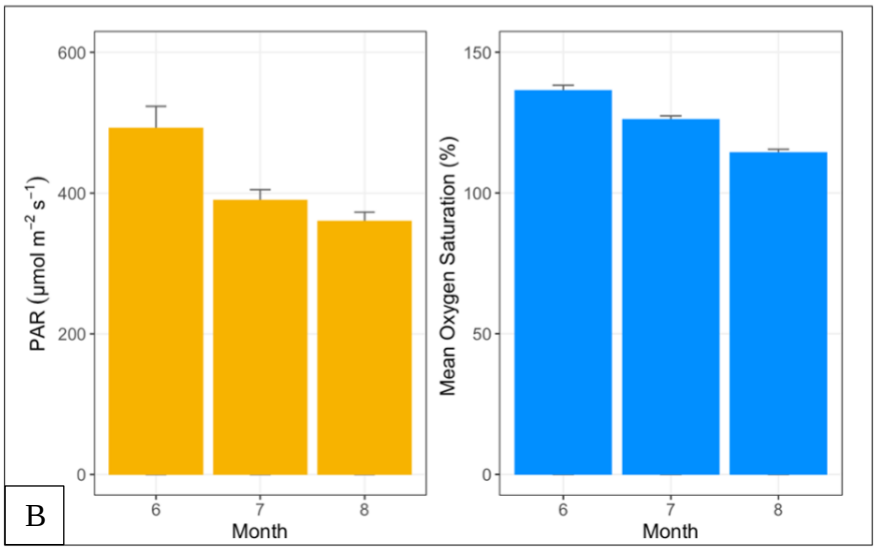

Figure 13. B) Mean PAR ± SE (n = June: 333, July: 1124, August: 1411) on the left and O_2 saturation ± SE (n = June: 333, July: 1124, August: 1411) on the right shown for each month. Only data from SB152 was used, as the North site was only sampled in July and August.

Gas fluxes for the artificial seagrass were compared with the concurrent fluxes for the natural seagrass (Figure 14). The ebullition rate for the artificial seagrass was significantly lower than the rate for the natural seagrass (p-value = 2.506*10^{-5}, t = -4.2871, DF = 276), and was practically zero. A similar comparison was conducted for the bare sediment experiment, with the results showing a difference that was not significant (p-value = 0.1176, t = -1.5733, DF = 163).

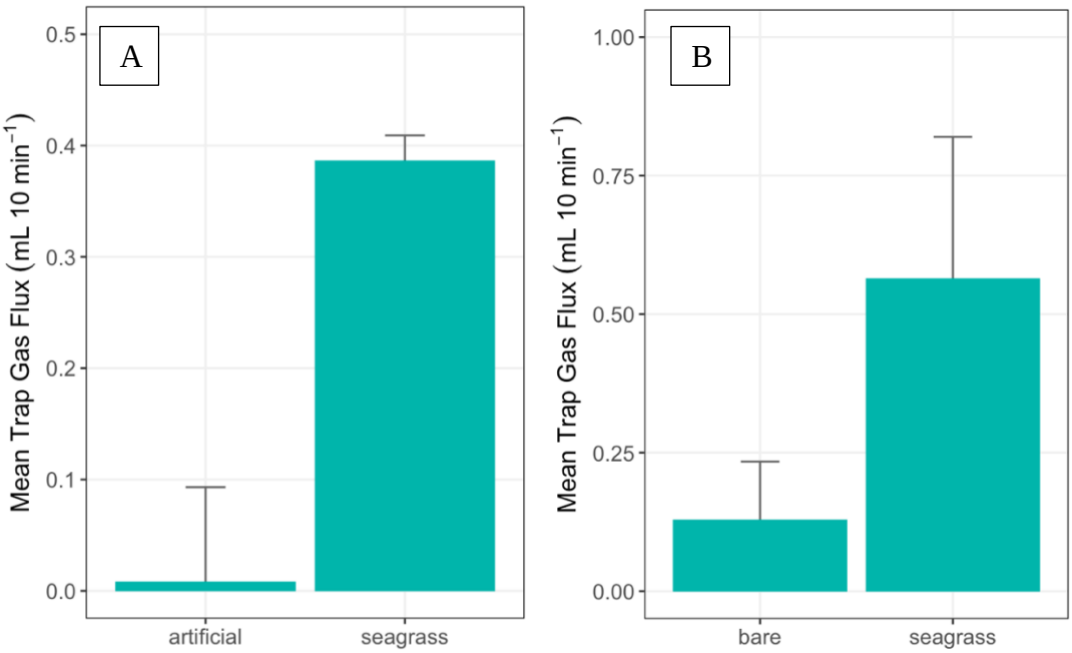

Figure 14. A) The mean 10-minute trap gas flux ± SE (n = 243) shown for the artificial mat traps compared to the natural seagrass traps during the artificial seagrass experiment. This experiment was conducted at the central site, SB152, for 10 days in the middle of July. B) The mean 10-minute trap gas flux ± SE (n = 132) shown for the bare sediment area compared to the natural seagrass during the bare area experiment that last a few days. No significant difference was found between the bare traps and the seagrass traps

The mean % volume of each gas measured (O_2, CO_2, CH_4) for the summer (July and August) was higher than the atmospheric standard % volume values. Analysis of the collected gas revealed differences in composition between sites (Figure 15). Based on the results of t-tests to compare % volume for each gas between SB152 and North, O_2, CO_2, and CH_4 % volume did not differ significantly between sites (O_2: p-value = 0.1716, t = -1.406, DF = 26; CO_2: p-value = 0.177, t = -1.4007, DF = 20; CH_4: p-value = 0.05813, t = 2.087, DF = 12). CH_4 flux, measured in μmol $m^{-2}d^{-1}$, did not differ significantly between sites (p-value = 0.2013, t = 1.3396, DF = 14). Both O_2 and CO_2 fluxes, measured in mmol $m^{-2}d^{-1}$, varied significantly between sites (O_2: p-value = 0.01105, t = -2.8626, DF = 16; CO_2: p-value = 0.002763, t = -3.2545, DF = 31). The mean O_2 bubble flux at SB152 was 4.2 ± 0.55 mmol O_2 m^{-2} d^{-1} in July and 5.1 ± 1.88 mmol O_2 m^{-2} d^{-1} in August. The mean O_2 bubble flux at the North site was 2.0 ± 0.59 mmol O_2 m^{-2} d^{-1} in July and 2.2 ± 0.40 mmol O_2 m^{-2} d^{-1} in August. The mean CO_2 bubble flux at SB152 was 0.52 ± 0.06 mmol CO_2 m^{-2} d^{-1} in July and 0.31 ± 0.03 mmol CO_2 m^{-2} d^{-1} in August. At the North site, the mean CO_2 bubble flux was 0.39 ± 0.08 mmol CO_2 m^{-2} d^{-1} in July and 0.16 ± 0.05 mmol CO_2

m^{-2} d^{-1} in August. For both CO_2 and O_2, gas fluxes were significantly greater at SB152. For CH_4, the mean bubble flux at SB152 in July was 0.70 ± 0.09 µmol CH_4 m^{-2} d^{-1} and was 0.45 ± 0.11 µmol CH_4 m^{-2} d^{-1} in August. The mean CH_4 flux at the North site in the form of bubbles was 0.35 ± 0.08 µmol CH_4 m^{-2} d^{-1} in July and was 1.23 ± 0.24 µmol CH_4 m^{-2} d^{-1} in August.

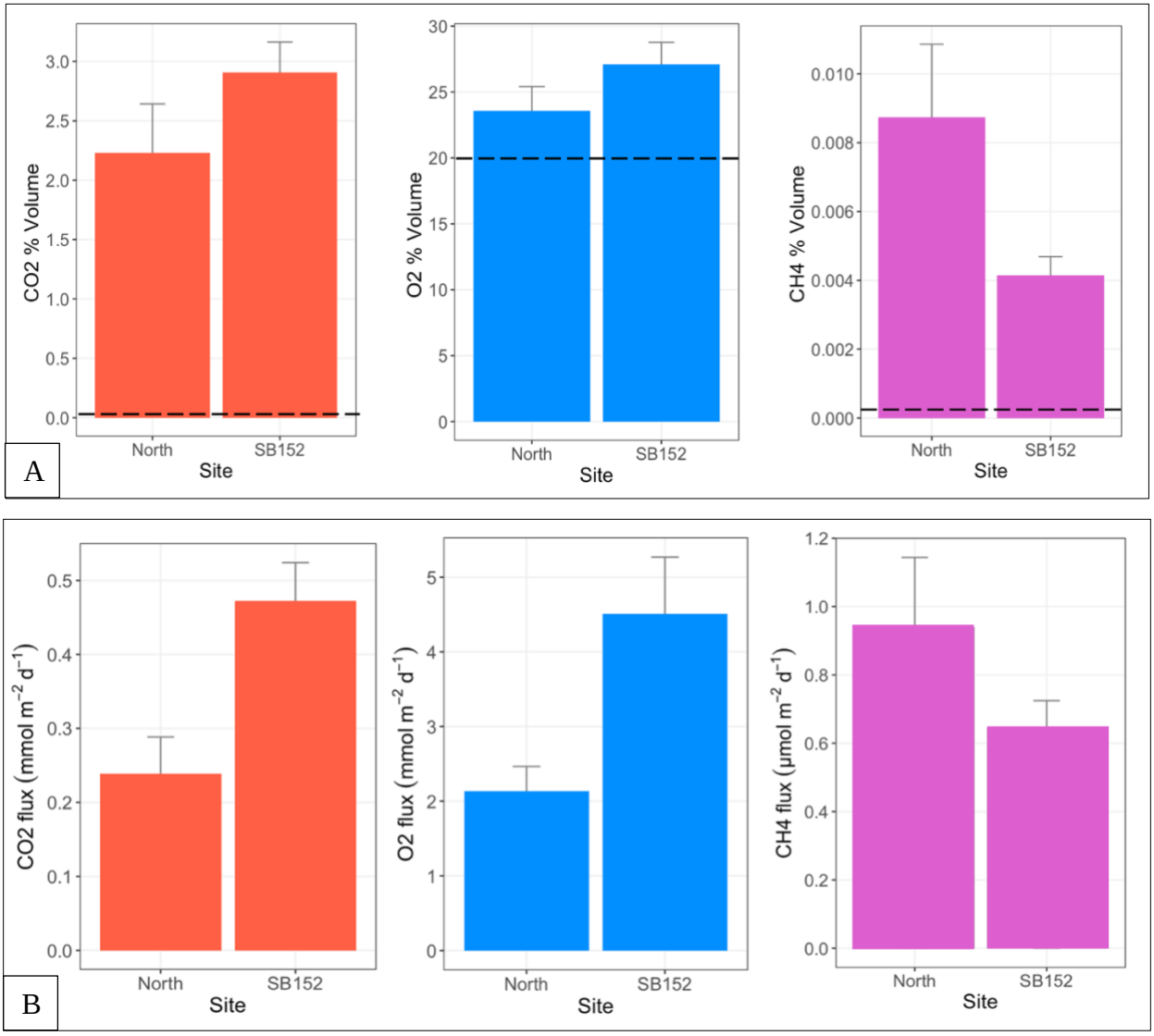

Figure 15. A) The mean summer (July and August) % by volume ± SE for each gas analyzed (CO_2, O_2, CH_4), with a comparison shown between sites. The black dashed line shows the approximate value of the standard atmospheric % volume for each of these gases. B) The mean daily flux ± SE of each gas (CO_2, O_2, CH_4), compared between sites. For both mean % by volume and mean daily flux O_2 n = North: 15, SB152: 13; for CO_2 and CH_4 n = North: 12, SB152: 24.

Discussion

In this study, we tested a novel, automated bubble trap design that employs off-the-shelf pressure sensors to estimate cumulative gas volumes. Testing results (Figure 5) showed that both laboratory and field experiments, where known amounts of gas were bubbled into the chamber at 5- or 10-minute intervals, had a high agreement between injected known volume and calculated volume. Mean values from lab and field tests showed a slope that was close to 1 (0.96 and 0.98) and had a high R^2 (0.99 and 0.98), indicating the high precision of the new method. During some field deployments that encompassed at least a full tidal cycle, however, an anomaly in readings was observed, most often around low tide. Laboratory and field tests suggested that the problem was caused by bubbles forming in the thin tubing between the collection chamber and the logger housing (Figure 3). The problem was solved by simply using tubing with a larger inner diameter. The new design was tested with bubble injection in the lab and in the field and showed no anomalies in calculated volume (see Supplementary Materials).

Using readings that did not contain anomalies, we calculated ebullition rates (as a 10-minute mean trap gas flux) to compare with concurrently measured environmental variables. An example deployment (Figure 6) shows the importance of using a mean value due to the variability between the individual traps, likely caused primarily by variations in the sampling areas. By averaging across multiple traps, we assess that we can account for most of the heterogeneity of the benthic surface due to meadow patchiness.

This study measured several environmental variables alongside the bubble trap deployments. Gas flux had a positive linear relationship with PAR and O_2 saturation, and a negative linear relationship with depth (Figures 8, 9, and 10). PAR has been shown to drive photosynthesis and thus O_2 production in seagrass meadows (Duarte et al., 1991; Rheuban et al. 2014b). O_2 gas was a significant component of the composition of bubbles collected, on average making up almost a third of the total volume of gas at our central site (Figure 15), up to 44% in some samples. Light and supersaturated O_2 conditions have been observed to be an important driver of bubble flux in other studies (Long et al., 2019; Lyimo et al., 2018).

As depth decreased, bubble fluxes increased in our study. This is in line with previous studies: low tides have been observed to increase ebullition (Long et al., 2019), as lower pressure conditions can enhance the formation of gas bubbles (Cozma et al., 2013). Low tide also corresponds with other covarying factors that create suitable conditions for bubble fluxes, such

as greater PAR reaching the top of the seagrass canopy and higher temperatures reducing gas solubility. Ebullition also had a clear trend in the time of day that it occurred, specifically with higher fluxes between 13:00 and 18:00, reaching a maximum in the late afternoon (Figure 11). Mean hourly O_2 saturation corresponded closely with the increase in ebullition in the afternoon, up to when mean hourly PAR dropped to very low levels towards the early evening.

A previous study in the VCR LTER seagrass meadows (Long et al., 2019) also found that ebullition is stimulated by high light intensity, high O_2 saturation, and low tides, with ebullition observed to be highest in the afternoon. The duration of this study was less than a week and sampling was done manually using conventional bubble traps. The study found that daily O_2 ebullition fluxes can be up to 11 mmol of O_2 m^{-2} d^{-1}, with a mean of 0.4 mmol of O_2 m^{-2} d^{-1}, and suggests that O_2 ebullition can be a significant component of total O_2 fluxes.

The multiple linear regression model using environmental variables (PAR, O_2 saturation, O_2 concentration, depth, water temperature, current velocity, and seagrass shoot density) to describe gas fluxes was not effective at predicting ebullition. The low R^2 value (0.073) indicates that the model only explains a small fraction of the variation in gas fluxes. This suggests that at least one key variable that drives bubble fluxes was not included and/or that some of these variables have highly non-linear relationships with ebullition. For example, seagrass shoot density can drive higher ebullition rates as greater densities increase overall meadow productivity (McGlathery et al., 2012). However, high shoot density also corresponds with increasing self-shading that reduces productivity (Pedersen et al., 2013). Temperature also could have a non-linear effect on gas fluxes. Higher temperatures would increase bubble formation as gas is less soluble in warmer water. However, *Zostera marina* productivity has been shown to decrease sharply past a certain temperature threshold as the seagrass experiences thermal stress (Berger, 2021; Staehr & Borum, 2011; Rasmusson et al., 2020). In addition to various non-linear relationships, it is also likely that the model does not include an essential environmental driver in bubble formation. Bubble formation on abiotic surfaces, as well as visual observations of highly varied bubble production under seemingly similar environmental conditions, suggests there are key physical drivers of ebullition that are not included among the variables measured here.

Gas flux varied substantially over the course of the summer. Ebullition was higher in June than in July or August at the central meadow site (Figure 12). It also varied spatially, with significantly lower gas fluxes observed at the northern site at the edge of the meadow (North)

compared to the central site (SB152). Both mean PAR and O_2 saturation were observed to be significantly lower at the northern site compared to the central site, likely due to North being deeper and having lower seagrass shoot density than SB152. PAR and O_2 saturation decreased at the central site over the course of the summer (Figure 13). The decrease in PAR over the course of the summer is likely due to higher counts of inclement weather in July and August, and/or increasing turbidity. The change in O_2 saturation over the course of the summer is most likely due to the increase in water temperature over time, reducing the solubility of O_2 in the water. As they both stimulate ebullition (Figure 8, 9) in the seagrass meadow, variations in PAR and O_2 saturation (Figure 13) could lead to both temporal and spatial variation in bubble flux.

Several studies assume that seagrasses and other benthic producers are forming non-dissolved O_2 through on-site photosynthesis, and methods have been developed to measure productivity while relying on this assumption (Felisberto et al., 2017; Wilson et al., 2012). Abundant formation of bubbles was observed on abiotic surfaces in the seagrass meadows of the VCR LTER (Figure 16), with similar bubble densities co-occurring on seagrass leaves and plastic surfaces.

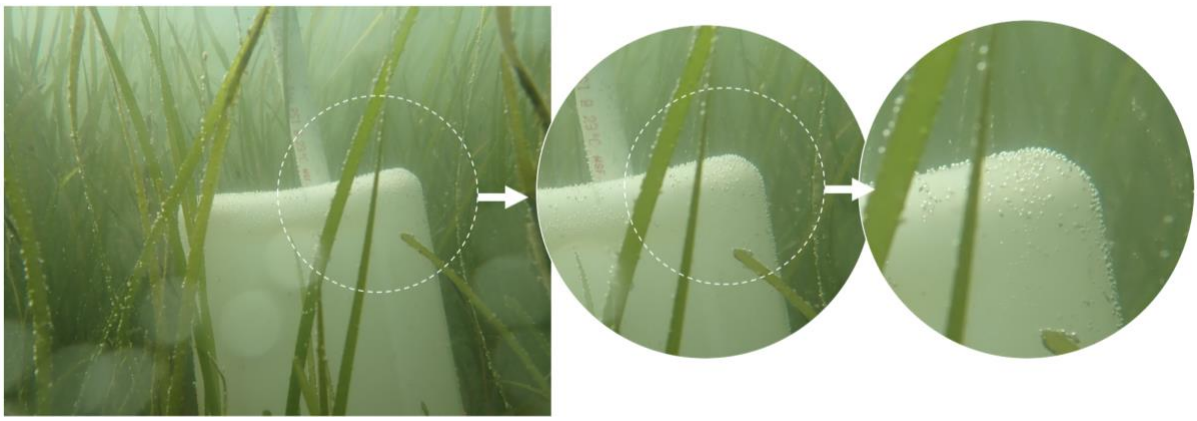

Figure 16. A time lapse photo of a plastic, abiotic surface recently submerged at SB152 in South Bay. Bubbles have formed at the same time of day on the leaves of the eelgrass, on the plastic surface, and on the camera lens. The magnified sections on the right show the high density of bubbles on the corner of the plastic surface.

To test whether bubbles are forming on leaves due to on-site photosynthesis, a field experiment was conducted using mats of artificial seagrass made from polypropylene film. We expected ebullition to be similar between the artificial seagrass bubble traps and the natural

seagrass bubble traps. However, the results of this experiment showed ebullition to be practically zero in the experimental traps (Figure 14), and not in line with the earlier observations of numerous bubbles on abiotic surfaces (Figure 16). We suspect that the material used for the artificial seagrass may not have been representative of the surface of a seagrass leaf, reducing the ability to support the nucleation of bubbles. Further study using bubble traps and artificial seagrass, maybe made with another material, is necessary to fully explain these diverging results.

The collected gas was analyzed for O_2, CO_2, and CH_4 composition (Figure 15). The % volume of O_2 was higher than atmospheric standard concentrations (20.9%) by several percent (Buis, 2019). The % volume of CO_2 and CH_4 in the collected gas was several orders of magnitude higher than standard volume percentages for atmospheric concentrations (CO_2 - 0.0407%, CH_4 - 0.00018%) (Buis, 2019). Between the northern site and the central site there was no significant difference in % volume for each gas, however, O_2 and CO_2 fluxes were significantly higher at the central site. These fluxes, reported as mmol (or μmol for CH_4) m^{-2} d^{-1}, were estimated from the sampled volume at the time of gas collection, the measured composition of the sampled gas, and the size of the trap sampling area. This allowed for the comparison of the bubble flux of O_2 to previously measured values for total O_2 fluxes using only dissolved O_2.

The results of our study, which had improved temporal resolution (10 minutes) and study duration (44 days of data collection over 3 months), gave O_2 bubble flux values of 4.2 ± 0.55 mmol O_2 m^{-2} d^{-1} in July and 5.1 ± 1.88 mmol O_2 m^{-2} d^{-1} in August at the central meadow site (Figure 15). Long-term measurements (2007-2018) at this same site using the aquatic eddy covariance technique have reported the mean Gross Primary Productivity (GPP) to be 170 mmol O_2 m^{-2} d^{-1} for July and 140 mmol O_2 m^{-2} d^{-1} for August (Berger et al., 2020). These values represent total dissolved O_2 flux. The addition of the mean O_2 bubble flux would only increase the estimated GPP value by 2.4% in July and 3.6% in August (Figure 17). It has been suggested that ebullition of O_2 could make up a considerable component of total O_2 fluxes (Long et al., 2019), but our much more detailed study shows that this is not the case. However, measuring these non-dissolved fluxes of O_2 could still provide value for understanding the metabolism of seagrasses and other vegetated coastal ecosystems. The seagrass meadow at the VCR LTER has been observed to be very close to the threshold between net heterotrophic and net autotrophic from metabolic measurements that only use dissolved O_2 fluxes (Berger et al., 2020), and incorporating estimates of O_2 ebullition may make a difference when a sharp boundary is used to

define ecosystem trophic status. Here, the mean annual value for Net Ecosystem Metabolism (NEM) has been found to be 1.0 mmol O_2 m^{-2} d^{-1} (Berger et al., 2020). Although the O_2 ebullition values found in this study are considerably higher than this, they still fall within the range of error for GPP estimates, as can be seen in Figure 18.

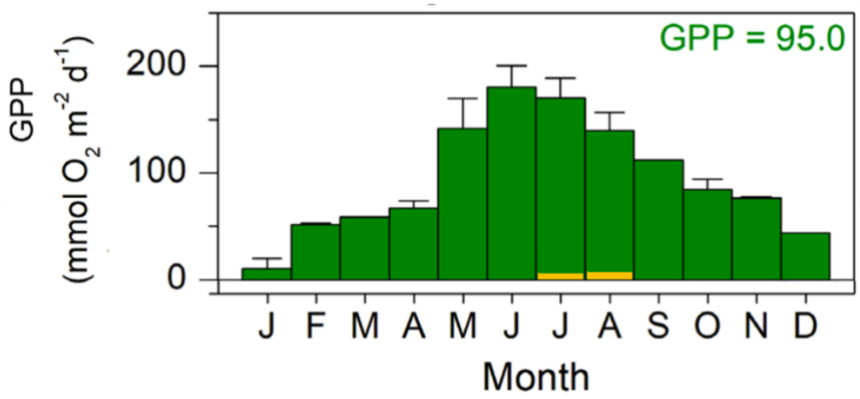

Figure 17. Mean monthly GPP for the central site (SB152) in South Bay from a decadal study spanning 2007-2018 and using the aquatic eddy covariance technique. Yellow bars show the mean values of mmol O_2 m^{-2} d^{-1} from ebullition fluxes measured in this study for comparison (modified from Berger et al., 2020).

Non-dissolved CH_4 fluxes (Figure 15) were 0.70 ± 0.09 µmol CH_4 m^{-2} d^{-1} in July and 0.45 ± 0.11 µmol CH_4 m^{-2} d^{-1} in August. A previous study in the South Bay meadow of the VCR LTER examined total CH_4 fluxes, finding a summer mean flux of 3.136 µmol CH_4 m^{-2} h^{-1} (Oreska et al., 2020). This flux included diffusive flux from bulk porewater measurements and ebullition measurements. Studies in other blue carbon ecosystems have found that ebullition contributes to 50% or more of total CH_4 flux (Chanton et al., 1989; Shalini et al., 2006). The highly variable nature of CH_4 fluxes, both spatially and temporally, suggest that further study is needed to understand the ebullition component of total CH_4 flux in these seagrass meadows. CO_2 ebullition has been largely unstudied in seagrass ecosystems, and this study was the first to our knowledge to examine seagrass CO_2 ebullition (Figure 15) over a longer sampling period. Supersaturation has been shown to occur in this meadow (Berg et al., 2019), and could likely result in CO_2 bubble formation during the nighttime when CO_2 concentrations are high.

A number of bubble trap studies have been conducted over a short period of time and sampled at a coarse temporal scale (Long et al., 2019; Oreska et al., 2020; Casper et al., 2000; Cheng et al., 2014; Keller & Stallard, 1994). Our study introduced a novel automated bubble trap design, build entirely from off-the-shelf components that allows sampling of gas volumes every

10 minutes throughout consecutive days and nights. The substantial variations in ebullition rates, which we still do not fully understand as documented here, underline the importance of this methodological advancement for future studies of gas exchange dynamics in coastal water. The work presented here points towards where some of these studies should be directed.

Supplementary Materials

In some deployments an anomaly was observed that typically occurs during falling tide and continues until the beginning of the rising tide (Figure 18). The thin tubing connection (ID 0.09 cm) between the collection chamber and the logger housing frequently was observed to contain bubbles during low tide events. These bubbles were evaluated to be the likely cause of the anomaly. For all data presented here, the data was visually assessed for the presence of these anomalies, and the data were excluded in further analysis. For example, for the case shown in Figure 18, data from the start of the deployment through approximately 07:30 were used along with data between approximately 03:00 to 07:00. Data following an anomaly was only used to describe the gas flux rather than cumulative volume as a conservative measure to ensure quality.

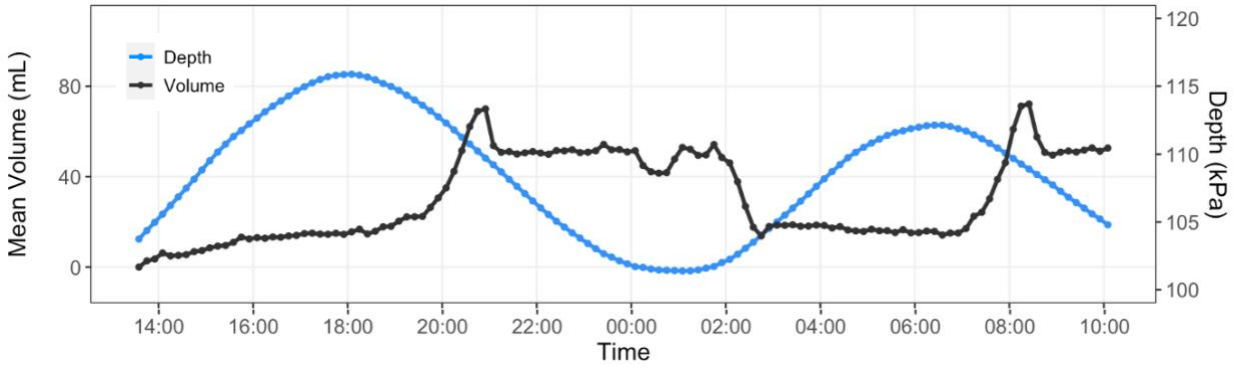

Figure 18. 10-minute cumulative volume readings for one bubble trap system from a deployment in July and depth readings (measured as pressure by the reference logger). The anomaly can be observed twice in this deployment: from slightly before 20:00 to slightly before 03:00 and from approximately 07:00 to the end of the deployment. The dip after midnight corresponds with when the top of the system came out of the water for a short period of time during the low tide.

To confirm that bubbles were producing these anomalous readings, laboratory and field tests were conducted where bubbles were purposefully allowed into the thin tubing (Figure 19). The results of both tests show the calculated volume from the pressure readings was not able to predict the known amount of gas that was bubbled into the collection chamber when bubbles were present. However, when the tubing was modified to have a larger inner diameter (0.39 cm) even when bubbles were observed in the tubing the calculated volume was determined correctly (Figure 20). The field tests through almost two tidal cycles (Figure 21) confirmed that the larger tubing did not produce anomalies in the readings. As a results, for future use of this bubble trap

design, we recommend using tubing with an inner diameter (repeat), and to perform further field tests under varying environmental conditions.

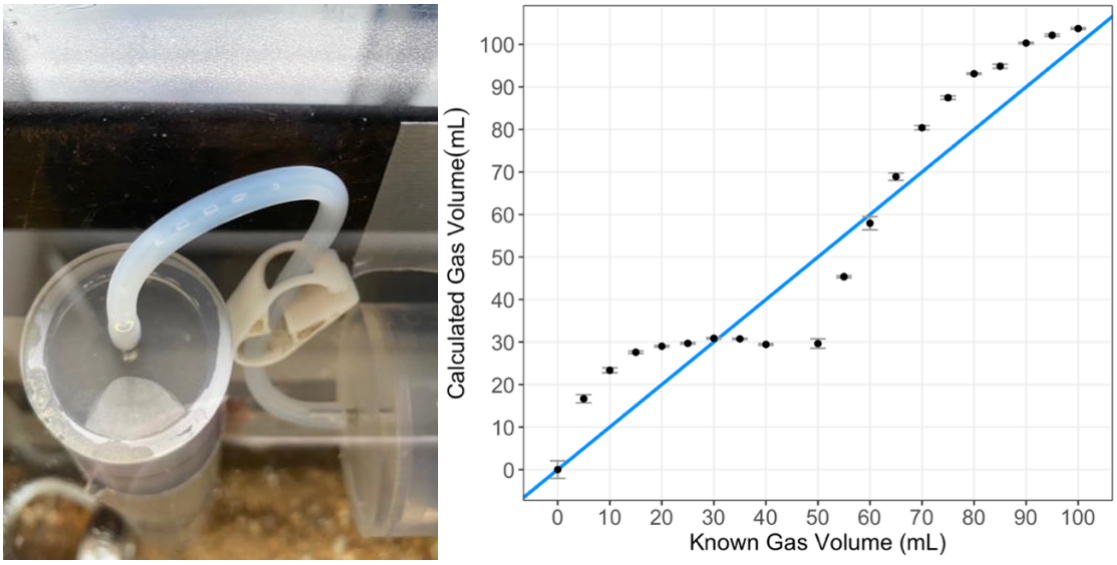

Figure 19. Using the original design of the system with the thin tubing (ID 0.09 cm), the system was set up in a laboratory tank and submerged. Bubbles were forced into the tubing. The subsequent calculated gas volume did not match the known gas volume that was injected into the collection chamber. The blue line here shows where the mean values of the calculated gas volumes should fall if those values accurately described the known gas volume.

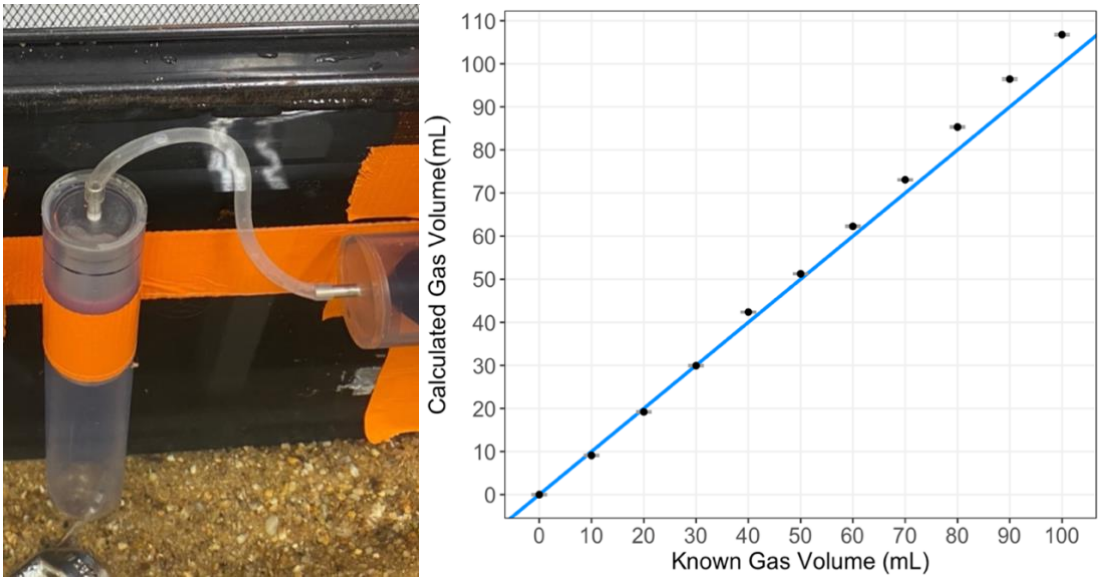

Figure 20. Using a modified design of the system with a thicker tubing (ID 0.39 cm), the system was set up in a laboratory tank and submerged. A bubble was forced into the tubing. The calculated gas volume matched the known gas volume that was bubbled into the collection chamber, similarly to when no bubbles are present in the thin tubing design.

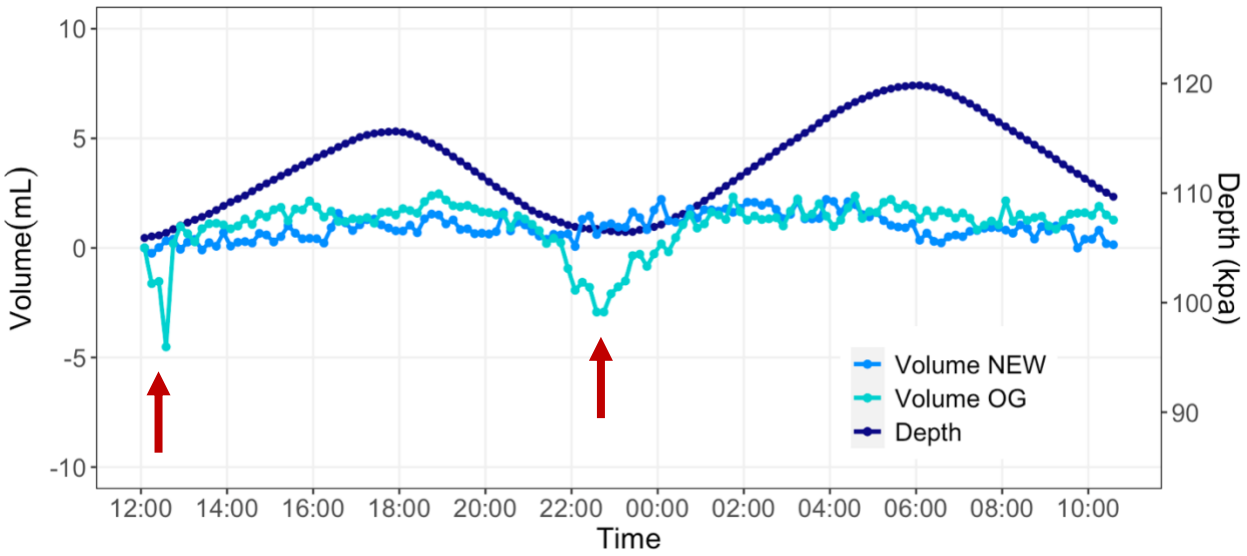

Figure 21. A field test where both the original design of the bubble trap system and the modified design of the bubble trap system were deployed, without the funnel attached. The volume measurements from the original design with the small tubing are shown as “Volume OG” and the volume measurements from the modified design with the larger tubing are shown as “Volume NEW”. Depth is shown to indicate the tide. In this test, bubbles were incorporated into the tubes of both systems and anomalies (indicated by red arrows) were seen at low tide in the original design with the small tubing, but not in the modified design.

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Conclusions

Seagrass meadows are highly productive ecosystems that offer many important services, including carbon sequestration and storage (Duarte et al., 2013; Fourqurean et al., 2012; McGlathery et al., 2007). To assess in situ carbon sequestration and storage, metabolic numbers including daily Gross Primary Productivity (GPP), Respiration (R), and Net Ecosystem Metabolism (NEM) are used along with other variables. These metabolic numbers are commonly estimated using measured benthic dissolved oxygen (O_2) fluxes (Berger et al., 2020; Odum, 1956). Metabolic estimates include several assumptions, two of which are explored in this thesis.

In the first chapter, we examined the assumption that respiration is constant and equal between day and night; an sumption used when calculating GPP and R. We examined trends in R from a long-term database of in situ O_2 flux measured by aquatic eddy covariance in a temperate seagrass meadow. To our knowledge, this was the first study of variations in R in a coastal ecosystem that used metabolic measurements performed under naturally varying environmental conditions. We found that R does vary between night and day and is not constant over a 24-hour period, decreasing by 29% over the course of the night. However, the linear nature of the variation allows for use of conventional calculations of GPP that assume that respiration is constant as long as a full 24-hour record of O_2 fluxes are used. This chapter highlighted the importance of long-term data to discern these trends in highly dynamic environments.

In the second chapter, we examined the widely used assumption that dissolved benthic O_2 fluxes for a seagrass meadow are representative of total O_2 fluxes and O_2 ebullition is negligible. To address this question, we developed a novel bubble trap design that uses highly accurate pressure sensors to quantify ebullition in aquatic systems. Because the system does not need to be manually sampled as traditional bubble trap systems, this design allows for data collection at a high temporal resolution (10 min) and longer deployments that are more representative of a whole season than previously performed measurements (Long et al., 2019; Oreska et al., 2020). This study found that O_2 ebullition makes up a small percent of the total benthic O_2 fluxes, only increasing GPP estimates by 2.4% in July, 3.6% in August. Our results also showed that high light availability, supersaturated O_2 conditions, and lower tides stimulated bubble fluxes from seagrass meadows. This study also indicates that bubble formation and release is more complex than generally thought. We have observed bubbles forming on abiotic surfaces, indicating bubble formation does not necessarily represent on-site photosynthesis on seagrass leaves.

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Appendix: VCR LTER Wind Study

Using long-term VCR LTER meteorological data to assess trends in coastal wind speed

Ieva Juska, Cora Johnston Baird, Peter Berg

Introduction

Local watermen and members of the community on the Eastern Shore of Virginia have noticed that small vessel fishing trips have been cancelled more frequently due to high winds, with older individuals remarking that they did not remember wind-caused cancellations ever occurring when they were young (Cora Johnston Baird and Albert Doughty, pers. commun., 2021). Researchers at the Virginia Coast Reserve Long-Term Ecological Research Site (VCR LTER) are also frequently affected by wind-caused small craft advisories that lead to research trip cancellations. Anecdotal evidence of increasing wind speeds from the community led to analysis of the VCR LTER long-term meteorological data (1991-2021).

Methods

Meteorological data at the VCR LTER is collected at two sites on the coast of the Delmarva peninsula (Oyster and Phillips Creek) and one site on a barrier island (Hog Island) (Figure 1). For this study, the Hog Island site was excluded from the analyses due to vegetation growth and site relocation causing significant year-to-year variability over the past three decades (John Porter, pers. commun., 2021).

Questionable readings and spikes in average hourly wind speed measurements were removed from the data prior to analysis. Average hourly wind speed was binned by year for both sites and for each of the two individual sites. The number of hours when average hourly wind speed was >6.7 m s^{-1} (15mph) was binned by year. At the VCR LTER, 6.7 m s^{-1} wind speed is the commonly used threshold to assess if conditions are safe for small research vessels. Linear regressions were performed for the yearly data and for un-binned data, and a significance level of 0.05 was used in all statistical tests.

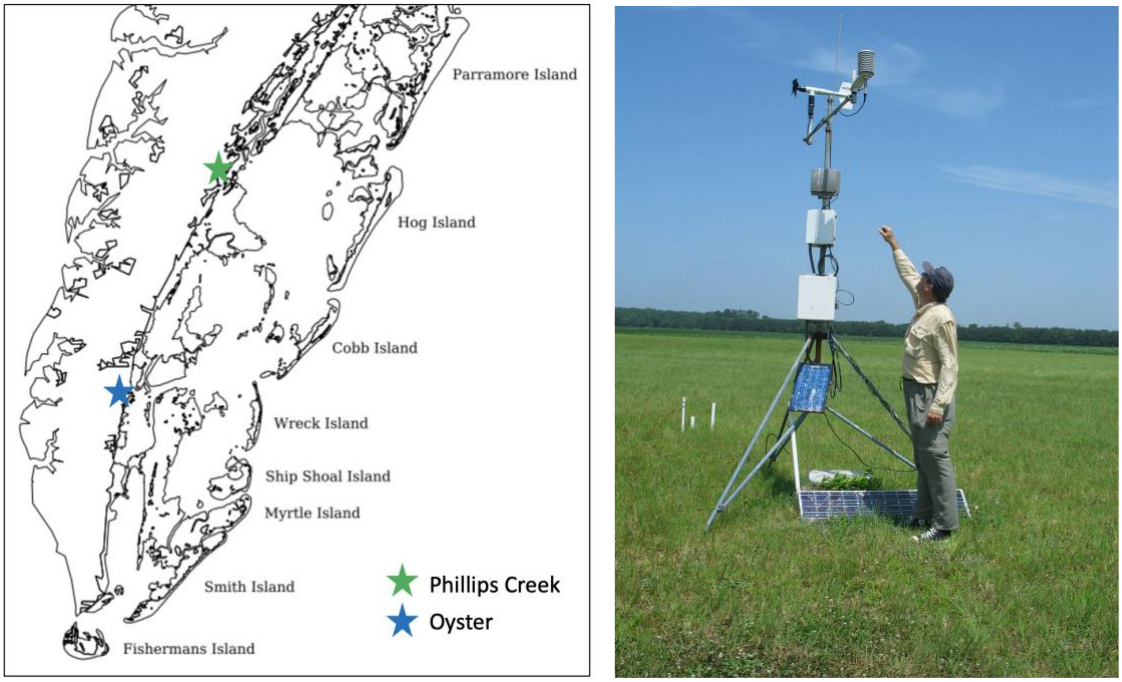

Figure 1. The location of the two coastal sites used in this study on the Eastern Shore of Virginia (left) and image of the meteorological station at the Oyster site (photo courtesy of John Porter).

Results

For Oyster, average wind speed had a positive relationship with year, and a linear regression of the binned data (Figure 2) gave the following results: a p-value of 0.03 and a R^2 of 0.15 (DF = 29). A linear regression with the un-binned data gave the following results: a p-value of $<2*10^{-16}$ and a R^2 of 0.008 (DF = 206526). For Phillips Creek, average wind speed also had a positive relationship with year, and a linear regression of the binned data gave the following results: a p-value of 0.002 and a R^2 of 0.33 (DF = 25). A linear regression with the un-binned data gave a p-value of $<2*10^{-16}$ and a R^2 of 0.006 (DF = 90600). For both sites combined, a linear regression of the binned data gave the following results: a p-value of 0.0006 and a R^2 of 0.34 (DF = 29). A linear regression with the un-binned data for both sites gave the following results: a p-value of $<2*10^{-16}$ and a R^2 of 0.01 (DF = 387735). Average hourly wind speed increased at each of the two coastal sites from 1991 to 2021.

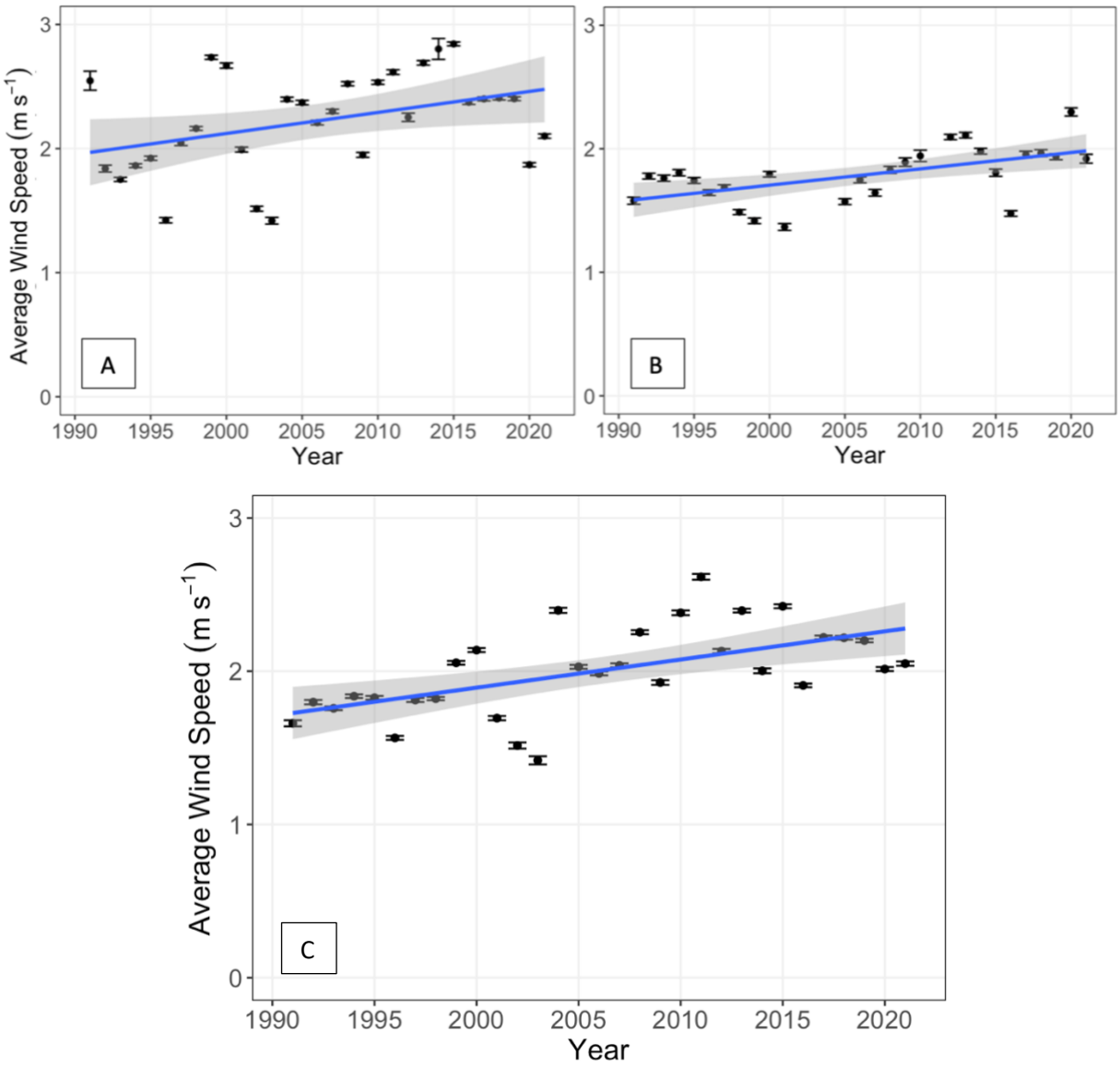

Figure 2. A. Oyster mean wind speed ± SE (n = mean: 6662, max: 8556, min: 201) B. Phillips Creek mean wind speed ± SE (n = mean: 3356, max: 4498, min: 1457) C. Coastal mean wind speed using data from both sites ± SE (n = mean: 12508, max: 17315, min: 4335). A linear fitting (± SE in gray) of the binned data is shown with a blue line in each panel.

The number of hours that average hourly wind speed was >6.7 m s^{-1} across both sites increased from 1991 to 2021 (Figure 3). A linear regression gave the following results: a p-value of 0.0008 and a R^2 of 0.33 (DF = 29).

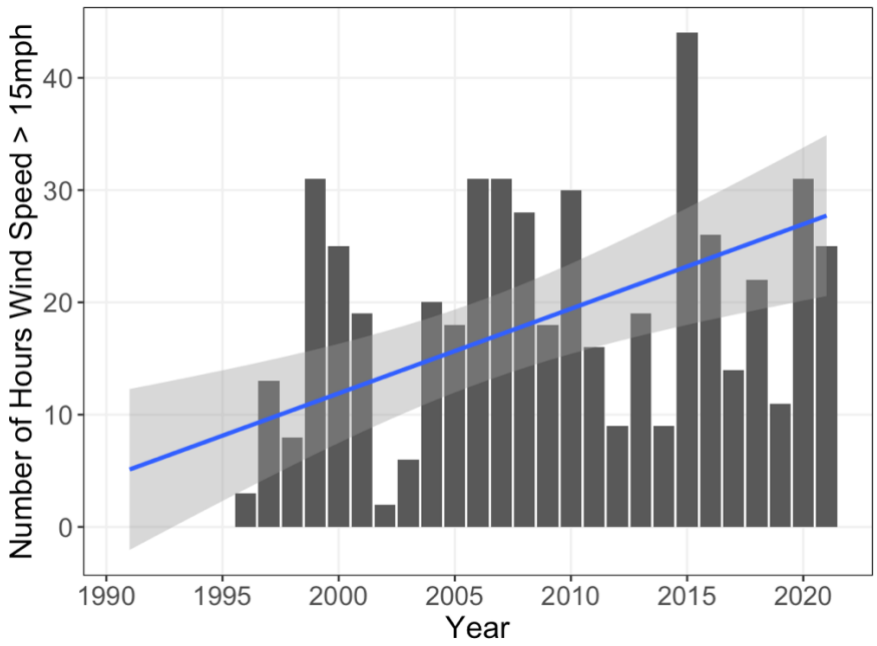

Figure 3. The number of hours that average wind speed was >6.7 m s^{-1} across both sites binned by year. A linear fitting (± SE in gray) is shown in blue.

Discussion

Mean coastal wind speed increased by approximately 0.5 m s^{-1} over the course of three decades, a 28.6% increase from 1991 to 2021. Both sites, Oyster and Phillips Creek, showed a very similar trend in wind speed between 1991-2021. A wind speed of >15mph is often used as a threshold for cancelling boating trips aboard small vessels at the VCR LTER. The number of hours when wind speed was above this threshold increased more than 5-fold at the two sites. This result generated from the VCR LTER long-term meteorological data (1991-2021) is in line with more comprehensive studies suggesting that anthropogenic climate change will increase wind speed over land (Eichelberger et al., 2008) and over the ocean (Young et al., 2011; Zheng et al., 2016), and that the rate of change will be greater for more extreme wind speeds (Young et al., 2011).

The local community members have noticed the higher incidences of wind-caused boat cancellations, which led to the analyses described here. This is a textbook example of co-production (Figure 4), where local knowledge and scientific knowledge come together to produce new and significant findings (Bremer and Meisch, 2017). In this case, the anecdotal evidence from the community drove the formulation of the hypothesis that led to the analyses of wind trends using the long-term quantitative data from VCR LTER scientists. The results from

this study are relevant to both scientists and the community, and directly relate to local concerns about wind-caused small vessel trip cancellations.

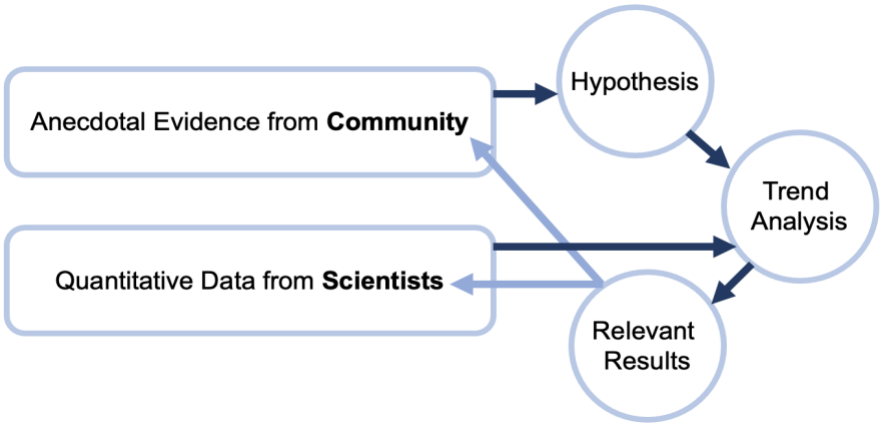

Figure 4. A conceptual diagram for the process of co-production in this study.

Future steps

As a future expansion of the work done in this study, collecting interviews from the local community about wind-caused cancellations would provide qualitative data to include alongside quantitative data from the long-term meteorological dataset. Examining the wind trends observed at other meteorological stations in the area, especially stations that have been collecting data for a longer period of time, could also provide a beneficial point of comparison.

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